UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA

LARYNGOPHARYNGEAL REFLUX, HELICOBACTER PYLORI AND INFLAMMATORY SINUS DISEASE

Paulo Jorge de Castro Borges Dinis

Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, Especialidade Otorrinolaringologia

Tese apresentada ao abrigo do regime especial de apresentação da tese, artigo 33.º do Decreto-Lei 65/2018, 1ª série, nº 157, de 16 de agosto

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CONTENTS

CHAPTER ONE

LARYNGOPHARYNGEAL REFLUX,

HELICOBACTER PYLORI

AND

INFLAMMATORY SINUS DISEASE

– IS THERE A LINK?

LARYNGOPHARYNGEAL REFLUX – AN OVERVIEW

In order to complete digestion, ruminating mammals have a physiological back and forth motion in their digestive track, which allows regurgitation of food back to the mouth for further chewing and mixing with saliva before the ingesta returns to the stomach.

In humans, however, the retrograde flow of the stomach contents into the esophagus is a non-physiological event attributed to a transiently compliant lower esophageal sphincter. Although this retrograde flow can occur up to 50 times a day, especially after meals, with no significant consequences as the esophageal mucosa is well prepared to intermittently contact with the acidic gastric contents, significant sustained exposure is known to be the cause of a chronic pathological condition named Gastroesophageal Reflux Disease (GERD). GERD is characterized by classic digestive symptoms such as heartburn and acid regurgitation and can lead to complications, e.g. esophagitis, esophageal strictures, and Barrett's esophagus. In the Western World it is estimated that between 10 to 20% of the population is affected by GERD, and its association to modern lifestyle habits such as the ingestion of certain foods (e.g. soft drinks, fatty foods, coffee, chocolate, spicy foods), alcohol consumption, smoking, obesity, and use of certain drugs has led some authors to consider GERD a modern life hazard.

Well known to gastroenterologists for decades, it was only in the 1980s that the groundbreaking work of Pellegrini and DeMeester, and of the Castell group, including Koufman,[1] who would later extensively investigate the subject, revealed the importance of reflux in airway pathology. Although occasional reports of reflux negatively impacting on the respiratory tract date back as earlier as the late 1960s, these authors showed, with use of a then new diagnostic tool – 24-hour double-probe esophageal pH monitoring

(with the second pH probe placed in the hypopharynx) –, that the backflow of gastric contents in humans can pass the upper esophageal sphincter all the way up to the upper aerodigestive tract and produce laryngeal and pharyngeal symptoms.[1] Koufman demonstrated, in an animal model, that single acid reflux events of short duration occurring three days weekly over two weeks were able to cause continuous inflammation of the respiratory mucosa.[1] This led to the reasoning that it would also take perhaps only as little as three reflux episodes per week to equally produce permanent inflammation of the respiratory mucosa in human supra-esophageal reflux disease.[1] In addition, pH-metry studies clearly showed that patients could experience several supra-esophageal reflux episodes in a 24-hour period, which, as single events, were usually asymptomatic and not identified as heartburn or as an acid regurgitation feeling.[1,2] Another major conclusion of Koufman's investigation was that acid-activated pepsin was the primary injurious component of the refluxate,[1,2] still showing capacity to inflict damage to the respiratory mucosa at pH values significantly higher than those in the stomach.[2] Later on it was revealed that, in addition to direct peptic-acid injury, vagus-mediated neuroinflammatory changes could also contribute to the pathogenic mechanism in supra-esophageal reflux disease.[2]

Subsequent studies have shown that the pattern of pharyngeal reflux is in many other ways different from classic GERD, with events occurring predominantly during daytime in the upright position in mostly non-obese subjects; by contrast, GERD is characterized by reflux episodes that usually occur in the supine nocturnal position in patients with an increased body mass index.[2]

In order to differentiate GERD from this supra-esophageal reflux disease, which presents with a variety of symptoms that gastroenterologists usually disregard such as cough, hoarseness, and globus sensation, a new term was coined – Laryngopharyngeal Reflux (LPR) – to replace outdated nomenclature such as atypical, occult or silent reflux, reflux laryngitis, and supra-esophageal reflux.[1,2] Dysfunction of the upper esophageal sphincter was suggested as its cause, and emphasis was put on the fact that heartburn and other digestive symptoms were absent in more than 50% of the cases (only 1/3 of the GERD cases overlap with LPR, and esophagitis is present in only 1/5 of the LPR cases), impelling clinicians to look for certain airway symptoms to consider this etiology.[2] A myriad of laryngeal and pharyngeal complaints have since been attributed to LPR, such as postnasal drip, recurrent pharyngitis, difficulty swallowing, globus pharyngis, chronic cough, chronic throat-clearing and intermittent hoarseness and other voice disturbances.[1,2] LPR has also been implicated in the etiology of chronic laryngitis, laryngeal carcinoma, subglottic stenosis, laryngeal granulomas, contact ulcers, and vocal nodules;[1,2] even chronic inflammatory sinus and ear diseases have been linked to LPR.[2]

LPR is currently believed to be one of the most important and common causes of inflammation in the upper airway, and it is, according to some, still often underdiagnosed and undertreated in the clinical setting, even by mindful otolaryngologists.[2]

Laryngoscopy is an important, easily performed test that is often employed in the diagnosis of LPR, but its results are fairly unspecific – erythema and edema of the posterior larynx have several other causes, and the test results are prone to subjective interpretation.[2]

The gold standard diagnostic tool for LPR, 24-hour esophageal pH monitoring,[1,2] is simply expensive, not widely available, and too unpractical to use in everyday clinical practice, inasmuch the current prevalence of LPR is estimated as having already reached epidemic proportions.[2] Moreover, dual-probe pH monitoring is still plagued by a lack of consensus regarding its methodology and does not provide a clear-cut division between those who suffer from LPR and healthy subjects since a number of individuals without symptoms also have laryngopharyngeal reflux.[2] Undoubtedly more people than it is clinically acknowledged have reflux reaching their laryngopharyngeal mucosa, and it is currently believed that the ones with symptoms are solely those who have a deficient mucosal protection mechanism against refluxate.[2] Indeed carbonic anhydrase type III, an enzyme that exerts an important epithelial protective function through active secretion of bicarbonate, a neutralizer of acid reflux, has been shown to be absent from the laryngeal mucosa in most people with LPR.

Response to acid suppression therapy with twice-daily proton-pump inhibitors (PPIs) is currently the most commonly used empiric diagnostic tool to confirm the diagnosis of LPR,[2] and some clinicians emphasize that symptoms usually improve before the laryngoscopic findings resolve. The failure of PPIs to ameliorate symptoms after a long enough period of treatment (opinions vary between 1 to 12 months) is unfortunately quite common,[2] which gives an idea of the complexity of the mechanisms involved. Clinically, it may be then necessary to either perform 24-hour pH testing (or test to detect pepsin in the hypopharynx) to confirm LPR, or to re-evaluate the initial diagnosis. Esophagogastroduodenal endoscopy may be useful to identify nonacid reflux (duodenal-gastric refluxate, featuring a high content of bile acids and pancreatic secretions), since in

those cases prokinetic agents may be a helpful adjuvant and Nissen fundoplication surgery may have to be considered.

Although previous research suggested otherwise, a recent study investigating the influence that coexistent *Helicobacter pylori* gastric infection can have on the success rate of LPR treatment with PPIs demonstrated that parallel eradication of the bacterium's gastric infection was able to significantly improve the efficacy of the PPIs in the treatment of LPR symptoms.

On the other hand, behavioral and dietary changes, empirically advocated for GERD treatment but lacking real substantive scientific support,[2] had their efficacy recently reassessed in the treatment of LPR, with some authors currently recommending a non-acid diet with alkaline water for those patients with LPR who are resistant to PPI therapy.

HELICOBACTER PYLORI – AN OVERVIEW

H. pylori is a helix-shaped gram-negative bacterium prevalent in the gastric contents of a large number of humans worldwide.[3] More than 50% of the world's population harbors the germ in their stomach, with prevalence rates as high as 80% in developing countries and in communities with poor socioeconomic status.[3] The infection it causes is asymptomatic in over 80% of the cases, and admittedly even plays a role in the natural stomach ecology.[3] Once infected, usually in early childhood, people will maintain lifelong *H. pylori* colonization until eradication by specific therapy or until gastric changes at an old age make the stomach inhospitable to colonization.[3]

Transmission is believed to be via the fecal-oral route and also, possibly, the oral-oral route.[3] When *H. pylori* reaches the stomach, a microbicidal environment to most species,

it manages to survive the extremely acidic pH by rapidly penetrating, through the active motion of its four to six flagella, the mucoid layer of the gastric mucosa, and by digging deep it attains the surface of the epithelial layer. Here, deep in the mucoid lining of the gastric mucosa with a more neutral pH milieu, the bacterium finds its niche in the human body, occasionally penetrating the epithelial cells, but mostly adhering to epithelial cells' external surface receptors.[3] The non-invasive mucosal surface attachment of the bacterium is, nevertheless, highly immunogenic, triggering a specific host response that involves neutrophils, T and B lymphocytes, plasma cells, and macrophages.[3]

Facing the permanent need of neutralizing gastric acid, *H. pylori* immediately engages in the local production of large amounts of the enzyme urease in order to create a basic milieu around itself.[3] Host tissue damage occurs when urease breaks down the urea normally found in the stomach to produce ammonia, which, together with other products of the *H. pylori* metabolism and potentiated by the acid and pepsin in the gastric lumen, has a toxic effect on the epithelial cells.[3] The two most well-known products of *H. pylori* metabolism responsible for increasing pathogenicity on the gastric epithelium are proteins CagA and VacA, and their production characterizes the more virulent strains of *H. pylori*.[3]

Although the infection is expressed clinically in highly variable ways, virtually all *H. pylori*-infected persons have chronic superficial gastritis, often displaying a patchy distribution at the stomach mucosal surface.[3] The germ's pathogenic capacity, however, depends on the interaction of several factors, including *H. pylori* strain-specific virulence factors (such as CagA and VacA), host susceptibility, environmental factors such as diet, and others.[3] If *H. pylori* gastric infection is generally asymptomatic, with the majority of

the infected individuals not developing clinical disease, there is a lifetime risk of developing peptic ulcer disease in 10% to 20%, stomach cancer in 1% to 2%, and gastric MALT lymphoma in less than 1%.[3] CagA and VacA production is usually low or absent in the *H. pylori* strains isolated from asymptomatic carriers of the germ, whilst the strains of *H. pylori* that produce high levels of these proteins are generally associated with the more pathogenic and symptomatic forms of the infection, including an increased risk of developing gastric cancer.

There is currently a wide consensus that there is no need to diagnose or even treat all cases of *H. pylori* gastric colonization, a virtually impossible task anyway since *H. pylori* gastric infection is the most widespread infection in the world and more than half of the world's population is infected.[3] Some authors even argue that *H. pylori* gastric colonization is part of the natural stomach ecology, hence more harm than good will likely result from eradicating the germ without necessity. The prolonged systemic immunologic response that *H. pylori* gastric colonization induces seems to benefit a number of diseases with an immunological component, henceforth the reported reduced prevalence of asthma, rhinitis, dermatitis, and inflammatory bowel disease in patients with *H. pylori* gastric infection.[3] Epidemiological studies have also shown that populations with higher rates of *H. pylori* gastric colonization have less severe GERD and lower incidence of esophageal adenocarcinoma, lending support to the thesis that *H. pylori* gastric colonization might somehow protect against reflux disease.[3] However, *H. pylori* eradication therapy has definitely been proven not to cause or exacerbate reflux disease, and the efficacy of PPIs in the treatment of reflux seems not to be altered by the patient's *H. pylori* status.[3] So it is currently agreed that testing and treating *H. pylori*

gastric infection should not be done routinely but only in specific circumstances: if peptic ulcer disease (active or not), atrophic gastritis or MALT lymphoma are present, after gastric cancer surgery, on first-degree relatives of patients with gastric cancer, and in certain cases of non-ulcer *H. pylori* positive dyspepsia. Another indication was recently added for treatment of *H. pylori* infection: patients who are on long-term treatment with PPIs and simultaneously have *H. pylori* gastric colonization seem to have a higher risk of developing atrophic gastritis, thus prophylactic *H. pylori* eradication may have to be considered in these patients.[3]

Invasive and non-invasive tests are used for the diagnosis of *H. pylori* gastric infection.[3] Non-invasive tests include the urea breath test, stool antigen tests, and serological tests (anti-*H. pylori* IgG, and anti-*H. pylori* CagA IgG antibodies). *H. pylori* can also be detected by histology, microbial culture, rapid urease test and polymerase chain reaction (PCR) tests on biopsied gastric mucosa samples collected during a gastroduodenal endoscopy.[3]

It has to be acknowledged that the sensitivity and specificity of each test varies (i.e. culture of the *H. pylori* organism from gastric biopsy material has the highest specificity but it is the least sensitive diagnostic test, whilst the other tests have a slightly lower specificity, but are more sensitive in various degrees) and that, with the exception of the serologic tests, false negative results may occur in patients who have taken antibiotics or omeprazole in the recent past. Consensual amongst researchers, however, is the fact that it is inappropriate to prescribe anti-*H. pylori* therapy without a firm diagnosis.

The 14-day “triple therapy”, which includes two antibiotics (clarithromycin and amoxicillin, or clarithromycin and metronidazole) and a PPI, is frequently recommended

as first-line treatment. Although other treatment regimens with different combinations of antimicrobials have been proposed, the "standard" triple PPI-clarithromycin and amoxicillin or metronidazole therapy is reported as progressively losing efficacy due to increasing antimicrobial resistance in certain parts of the world.[3]

LARYNGOPHARYNGEAL REFLUX, H. PYLORI AND INFLAMMATORY DISEASE OF THE UPPER AERODIGESTIVE AND RESPIRATORY TRACT

LPR and Otitis Media

As soon as it was recognized that LPR could be linked from an etiopathogenic perspective to laryngeal and pharyngeal pathology, several reports emerged, incriminating LPR in disease processes even higher up in the respiratory tract, such as in the nose and sinus and in the middle ear.

Tasker *et al.*[4] in 2002 were among the first to report they had found pepsin/pepsinogen concentrations levels up to 1000 times higher than serum levels in middle ear fluid from children with otitis media with effusion, which led to the conclusion that the gastric juice seems to play a role in its etiopathogenesis.

Since then, other authors repeated the same observation with varying pepsin/pepsinogen values.

Miura *et al.*[5] recently conducted a meta-analysis of the literature on the association between otitis media and gastroesophageal reflux, selecting only 15 out of 242 papers as suitable for inclusion. From those data they concluded that the reported prevalence of GERD in children with chronic otitis media with effusion or recurrent acute otitis media

is indeed significantly higher than the prevalence of GERD in the general pediatric population: the mean prevalence of GERD in children with chronic otitis media with effusion is 48.4% (range, 17.6%-64%), and in children with recurrent acute otitis media it is 62.9% (range, 61.5%-64.3%). The mean prevalence of LPR in children with otitis media is 48.6% (range, 27.3%-70.6%), whereas the mean middle ear pepsin/pepsinogen presence in otitis media is 85.3% (range, 60%-100%) and enzymatic activity is 34.2%. The authors stress, however, the fact that the two sole randomized studies in the literature on the efficacy of PPIs in otitis media treatment could not find a significant benefit.

LPR and Sinusitis[1]

Chambers *et al.* [6] evaluated the predictive objective factors of poor outcomes after endoscopic sinus surgery and were somehow surprised to find that GERD, on par with the post-operative scarring of the ostio-meatal complex region, was the most critical variable.

Bothwell *et al.*[7] prescribed anti-reflux therapy to 28 children selected for sinus surgery due to recalcitrant chronic rhinosinusitis (CRS) and reported that only 3 still required surgery at the end of the treatment. They concluded that reflux should always be assessed and treated before sinus surgery is considered in children.

Catalano *et al.*[8] assessed, using gastroesophageal endoscopy, the prevalence of esophagitis in 110 adult patients with no digestive symptoms but suffering from various upper respiratory tract pathologies (posterior laryngitis, vocal fold nodules and chronic sinusitis). In comparison to 117 subjects undergoing gastroesophageal endoscopy for

[1] **Submitted** for publication to American Journal of Rhinology & Allergy

other reasons than GERD, they found that the patients with those upper airway disorders have a significantly higher prevalence of esophagitis than controls (31% versus 15.4%).

Ulualp *et al.*,[9] in a controlled study employing ambulatory 24-hour double-probe esophageal pH-metry (with the second pH probe placed in the hypopharynx), showed that adults with refractory CRS had a significantly higher prevalence of pharyngeal acid reflux episodes.

DiBaise *et al.*[10] compared, using 24-hour double-probe esophageal pH-metry, the reflux profile of CRS patients with that of patients diagnosed with GERD, and reported that the pH test results of both groups were similar. However, when these sinusitis patients undertook a 3-month trial treatment of omeprazole b.i.d., the clinical results showed only modest improvement.

Katle *et al.*[11] had a Sino-Nasal Outcome Test 20 (SNOT-20), a nose- and sinus-related quality of life assessment questionnaire, submitted to one group of GERD patients and to a control group. They found out that the average total SNOT-20 score in patients with GERD was 22.1, and in the control group 9.4, meaning that that GERD patients have a reduced nose- and sinus-related quality of life compared to controls.

Bohnhorst *et al.*[12] followed a similar approach, using the SNOT-22 test (a survey instrument evaluating the severity of sinonasal symptoms associated with chronic rhinosinusitis) in a group of GERD patients and in the general population to find out that the prevalence of CRS among patients with GERD was 20.7%, compared to 8.5% from the general population.

DeConde *et al.*[13] administered three specific quality-of-life questionnaires (the SNOT-22, the Rhinosinusitis Disability Index, and the Chronic Sinusitis Survey) to 229 adult

patients with CRS before sinus surgery and at 6, 12, and 18 months postoperatively, and compared the results to those from patients with (n=72) and without (n=157) history of GERD. They could not demonstrate that history of GERD co-morbidity increased the burden of CRS symptoms on quality-of-life, either for baseline characteristics or outcomes following surgery.

Delehaye *et al.*[14] evaluated 50 reflux patients undergoing gastroesophageal endoscopy, having them fill out a SNOT-20 questionnaire and perform a saccharin nasal mucociliary clearance test. A total of 74% subjects had a prolonged saccharin clearance time (23.79±5.58min), corresponding to the subset of patients who also had significantly higher, although within normal range, mean SNOT-20 scores (19.3), and presented more pathology during gastroesophageal endoscopy, with typical GERD complaints. The remaining 26% patients, with normal saccharin clearance times (8.15±2.06min), constituted another subset of subjects, who tended to have lower mean SNOT-20 scores (7.4), as well as less pathology on gastroesophageal endoscopy, and atypical, extraesophageal, reflux complaints.

A different conclusion was reached by Durmus *et al.*,[15] who also investigated if reflux disease is associated with nasal mucociliary transport changes. They performed a saccharin nasal mucociliary clearance test on 50 patients with GERD and/or LPR, and their results were compared to those of a control group with 30 healthy subjects. The study group underwent an additional saccharin nasal mucociliary clearance test after a 12-week period of treatment with PPIs. They concluded that the study group's mucociliary clearance time was within the normal range at all time points.

DelGaudio,[16] using triple probe pH-metry with sensors at both the hypopharynx and nasopharynx, demonstrated that subjects with persistent CRS after endoscopic surgery had significantly higher rates, compared with controls, of both GERD and LPR, the later including reflux episodes detected at both hypopharynx and nasopharynx sensors.

Wong *et al.*[17] used 24-hour four-channel pH-metry with sensors at the distal and proximal esophagus and at the hypopharynx and nasopharynx to find that 32.4% of the adult CRS population evaluated had reflux, with 23.1% of the detected episodes reaching the proximal esophagus, 3% reaching the hypopharynx, and only 0.2% reaching the nasopharynx. They concluded that mechanisms other than a direct contact of the refluxate with the nasopharyngeal mucosa were probably at stake in the association between reflux and chronic rhinosinusitis.

The same group[18] challenged the lower esophagus of 10 volunteers without GERD or sinusitis with an acid infusion and observed an increase in nasal mucus production and general nasal symptom scores, which returned to baseline 45 minutes after the challenge. According to the authors, these results support the thesis of a neural reflex between the esophagus and the sinuses, via the vagus nerve, which would contribute to sinusitis pathogenesis in patients with GERD.

Loehrl *et al.*[19] had 20 patients with CRS undergo several LPR diagnostic tests at the same time. They concluded that while pH-metry showed reflux reaching the pharynx in 95% of the patients, the corresponding nasopharynx results correlated poorly with the pharyngeal results. Nasopharyngeal tissue biopsies performed to identify the presence of pepsin were negative in all subjects. However, a pepsin identification test performed on the nasal

lavage fluid showed positive results, a sure sign, according to the authors, that there is in fact direct contact of the refluxate with the sinonasal mucosa in these patients.

Ozmen *et al.*[20] confirmed that a pepsin identification test of the nasal lavage fluid seems to be a reliable tool to identify LPR in patients with CRS by showing 100% sensitivity and 92.5% specificity in comparison to 24-hour dual-probe pH monitoring. In their series of cases, 33 CRS subjects showed a higher rate of hypopharyngeal acid reflux events than 20 non-CRS subjects (88% versus 55%), and the fluorometric pepsin assay they used proved to be a reliable noninvasive LPR screening method, alternative to pH monitoring (all their patients with intranasal pepsin had hypopharyngeal reflux).

Phipps *et al.*[21] studied children with refractory CRS using 24-hour double probe esophageal pH-metry and demonstrated that they have a much higher prevalence of GERD than the general pediatric population, with 32% clearly showing evidence of reflux in the nasopharynx. They pointed out that sinus disease improves for most of these children after treatment for GERD.

Pincus *et al.*[22] selected 30 adult patients with recalcitrant CRS and performed 24-hour either double or triple probe esophageal pH-metry, which showed reflux disease in 25 of them. These patients were started on a PPI, and after one month most of them had seen improvements in both their GERD and sinus symptoms.

Jecker *et al*.[23] compared the 24-h double probe pH testing data of 20 patients with nasal polyps with the data of 20 controls. Surprisingly, they found that the number of reflux episodes registered at the esophageal sensor was 10 times higher in the polyps group than in the controls, but the number of reflux events at the nasopharyngeal sensor was about the same in both groups.

A not very different conclusion was reached by Shaker *et al.*,[24] who, using concurrent dual pharyngeal/dual esophageal pH-metry, found that none of the two groups of patients with suspected LPR pathology (reflux laryngitis and vasomotor rhinitis) had any significant difference in number, duration and pattern of pharyngeal acid refluxate events when compared to a control group. In realizing that asymptomatic controls also have pharyngeal acid reflux to a certain degree, these authors formulated the hypothesis that perhaps local protective factors in the airway mucosa hold the key in determining which individuals with reflux will develop symptoms.

Wise *et al.*[25] studied specifically the subgroup of patients who have persistent post-nasal drip as their most distinctive complaint, and used 24-hour triple probe pH-metry (sensors at the esophagus, laryngopharyngeal region and nasopharyngeal region) to evaluate three groups of patients: 1) patients who had undergone sinus surgery and kept complaining of post-nasal drip; 2) patients undergoing sinus surgery who were happy with their surgical results; and 3) volunteers without sinusitis. They concluded that all subjects with objective evidence of laryngopharyngeal and nasopharyngeal reflux at the pH<5 threshold had post-nasal drip complaints and suggested reflux treatment for them.

Leason *et al.*[26] recently completed a meta-analysis study of published articles on reflux and CRS in the English language. They concluded that the data unequivocally support a significant association between reflux (both acid and non-acid) and CRS. The published evidence in the medical literature mostly supports a pathogenic role for reflux in CRS, with sinusitis patients having greater prevalence of intranasal *H. pylori* and acid reflux than subjects without the disease, and with chronic rhinosinusitis being more prevalent in those with reflux than in those without.

H. pylori, Tonsils and Adenoids

Unver *et al*.[27] found a high rate of *H. pylori* colonization in tonsil and adenoid tissues after studying adenotonsillectomy specimens of children and young adults using a Campylobacter-like organism (CLO) test.

Cirak *et al*.[28] used PCR to detect the presence of *H. pylori* DNA in 30% of the adenotonsillectomy specimens of children, with most of them (71%) also possessing the CagA gene.

Bulut *et al*.[29] reached similar conclusions, with 24.6% of adenotonsillectomy specimens from children being positive for *H. pylori* by PCR, 58.6% of which were also positive for the cagA gene, the latter a specific feature seemingly associated with adenotonsillar hypertrophy.

Lin *et al.*[30] investigated *H. pylori* colonization in two groups of patients with different causes for the tonsillectomy: one group had tonsillectomy for chronic recurrent tonsillitis and another group for sleep-related breathing disorders. They encountered different *H. pylori* colonization patterns according to the indication; the tonsillitis group showed 48% *H. pylori* positive colonization and the sleep-related breathing disorders group only 24% of *H. pylori* positive colonization.

Nártová *et al.*,[31] in a group of adults with chronic tonsillitis and sleep apnea syndrome, encountered 80% of positive results for *H. pylori* by PCR in the tonsils of patients in the tonsillitis group, with the cagA gene detected in 25% of them, whilst the tonsillar tissue of the patients in the sleep apnea syndrome group had 82.7% positivity for *H. pylori* with 20.8% of cases positive for the cagA gene. These findings led them to conclude that *H. pylori* presence in

the tonsils could be an etiopathogenetic factor in chronic tonsillitis and tonsillar hyperplasia related to sleep apnea.

Pitkaranta e*t al.*,[32] on the other hand, were unable to culture *H. pylori* from either adenoid tissue or middle ear fluid collected from children undergoing adenoidectomy and/or tympanostomy tube surgery, in spite of the fact that some of these children tested positive for *H. pylori* on serologic and fecal antigen detection tests.

Yilmaz *et al.*[33] investigated *H. pylori* presence using the CLO test in adenotonsillectomy specimens from 50 children, and found that they all tested negative despite positive results for *H. pylori* stool antigen and/or serum *H. pylori* IgG antibody for most of the children.

Moreover, Di Bonaventura *et al.*,[34] when evaluating tonsillar *H. pylori* colonization by performing a bilateral tonsillar swab in a group of patients who were undergoing gastroduodenal endoscopy for dyspepsy, with 58.3% of them having documented gastric *H. pylori* infection, did not find a single positive result for the bacterium in both the microbiological culture and immunochemical analysis.

Jelavic *et al.*[35] selected the adenotonsillectomy specimens in their series that had tested positive in the rapid urease test (17/139) and had them cultured, but none of them grew *H. pylori.* They therefore concluded that adenotonsillar tissue does not seem to be a reservoir for *H. pylori.*

Katra *et al.*,[36] using PCR, evaluated the presence of *H. pylori* in adenoid tissue of children selected for adenoidectomy who presented simultaneously LPR-suspected symptoms, and concurrently assessed for LPR by pH monitoring, with the proximal sensor placed 1 cm above the upper esophageal sphincter. They found that the children

with *H. pylori* in their adenoid tissues had significantly more reflux episodes reaching their pharynx, a fact supporting the thesis that LPR episodes play an important role in *H. pylori* presence in the adenoid tissue.

Lukeš *et al.*[37] had tonsillar tissue collected from adult patients with either chronic tonsillitis, obstructive sleep apnea syndrome or tonsillar carcinoma assessed for *H. pylori* presence by culture and PCR. They found that the presence of the bacterium in the tissues was highly prevalent for all three pathologies, being detected in 73.91% of tonsillar tumors, in 70.0% of tonsillitis cases, and in 69.23% of obstructive sleep apnea syndrome specimens. More importantly, the gene analysis of virulence factors showed differences in the strains found in the oropharyngeal lymphoid tissue in these pathologies compared with the strains most commonly found in the stomach, with the former showing lower abundance of the cagA gene and presence of the less virulent vacA gene allele combination. This suggests that the *H. pylori* strains in the oro-pharynx of these patients could have a genotype different from that of the strains found in their stomachs.

Bitar *et al*.[38] compared three methods (rapid urease test, histology identification and PCR) for the evaluation of presence of *H. pylori* in children's adenoid tissue, and reported that: 1) there was a high number of false positive results when the rapid urease test was used to diagnose *H. pylori* presence (84% samples tested positive); 2) histologic identification seems unreliable to detect *H. pylori* in an extragastric location (16% samples showed *H. pylori*-like microorganisms); and 3) PCR seems to be the best way to identify the germ correctly (all the samples tested negative by PCR). These observations allowed them to conclude that perhaps *H. pylori* is not present in the adenoid tissue after all, and previous authors have misdiagnosed its presence owing to inappropriate methodology.

Abdel-Monem *et al.*[39] estimated that the rapid urease test has a sensitivity of 100%, but a specificity of just 56%, whereas PCR has a sensitivity and specificity of 100%, although 16.6% of the population in their study tested positive for *H. pylori* in adenotonsillar tissue by PCR.

Jabbari Moghaddam *et al*.[40] compared the rapid urease test and histology in their capacity for identifying *H. pylori* in tonsil biopsy samples and concluded that the former was perhaps not sensitive enough as 39.6% of the samples tested positive by histopathology and only 14% had a positive rapid urease test.

Najafipour *et al*.[41] also found a poor agreement between the results from the rapid urease test and PCR in the detection of *H. pylori* in tonsil biopsy samples, reporting 48.5% of positive results with the former and 19.4% with the later.

From the same group, Farivar *et al*.[42] also reported poor agreement between PCR and histology in identifying *H. pylori* in tonsil biopsy samples, with 21.4% positive with the former and 18.4% positive with the latter method.

By contrast, Aliakbari *et al*.[43] found that, in adult patients with a diagnosis of chronic tonsillitis and adenoid hypertrophy, not a single one of them had a positive PCR test for *H. pylori* in adenotonsillar tissues, although 65% were found to be seropositive for *H. pylori* IgG and 8% presented digestive symptoms. In addition, neither seropositivity nor digestive symptoms correlated with adenotonsillar *H. pylori* colonization.

Khademi *et al*.[44] analyzed by CLO the *H. pylori* colonization pattern in tonsillar surface tissues and tonsillar core tissues and concluded that while *H. pylori* was present in 53% of the surface samples, the bacterium was only present in 23% of the core samples, with solely 12% of the studied tonsils having *H. pylori* present in both the surface and core.

The authors thus warned that all *H. pylori* results obtained from the tonsil surface may not represent the tonsil core.

A different conclusion was reached by Aslan *et al.*[45] who found no differences in the *H. pylori* colonization pattern between tonsillar surface (42%) and core (47%) tissues using the Pronto Dry test. They further performed histopathology staining in the tonsillar samples to detect the expression of inducible nitric oxide synthase in macrophages of the tonsils, and concluded that its activity was significantly higher in the biopsy tissues that also tested positive for *H. pylori*, which led them to conclude that *H. pylori* could promote tonsil inflammation, if not as a causative agent then at least as a promoter of the inflammatory response by triggering macrophage activity.

Skinner *et al.* [46] also investigated if *H. pylori* was associated with increased expression of inducible nitric oxide synthase in macrophages of the tonsil, and they indeed detected an increase in the number of macrophages stained for this enzyme in this tissue; this result related to systemic *H. pylori* seropositivity only, as all their tonsil tissue samples were negative for *H. pylori*.

Kusano *et al*.[47] demonstrated that *H. pylori* can be present in the palatine tonsils crypts in the coccoid form. In their series of tonsillar samples from 55 patients with recurrent pharyngotonsillitis or IgA nephropathy, 78.2% were positive for tonsillar *H. pylori*, identified by immunofluorescence and immunoelectron microscopy using antibodies against *H. pylori*. Most samples (88.4%) were positive for the cagA gene, and 27.3% of the patients were shown to have *H. pylori* gastric infection, all of whom concurrently had *H. pylori* in their tonsils.

Minocha *et al.*[48] studied patients undergoing gastro-duodenal endoscopy for varied reasons and identified two groups, those with and those without *H. pylori* gastric infection. By scrutinizing the patients' background, they found out that a prior tonsillectomy was performed in 30.6% of the subjects in the *H. pylori*-negative group versus 5.4% of the subjects in *H. pylori*-positive group. They then concluded that a history of tonsillectomy is apparently associated with decreased prevalence of *H. pylori* gastric colonization, thus supporting the theory that tonsillar tissue is a reservoir for *H. pylori* infection.

Opposite conclusions were, however, reached by Sezen *et al.*,[49] who conducted a prospective study to determine the effect of tonsillectomy on the eradication of *H. pylori* from the gastrointestinal tract. Patients with *H. pylori*-positive gastric infection were divided into three groups: 1) those who first underwent tonsillectomy and afterwards received combination-drug therapy for 14 days; 2) those who first had the same treatment regimen and then underwent tonsillectomy; and 3) those who just received the drug treatment. The success of *H. pylori* eradication was assessed by gastroscopy after two months. Similar success rates were encountered in the three groups (75%, 80% and 70%, respectively), allowing the authors to conclude that tonsillectomy has no significant effect on gastric *H. pylori* eradication and that tonsillar tissue does not seem to be a reservoir for *H. pylori* infection.

H. pylori and Otitis Media

Pitkaranta *et al.*[50] failed to grow *H pylori* after culturing both middle ear fluid and adenoid tissue collected from children undergoing tympanostomy tube placement and

adenoidectomy for chronic otitis media with effusion, although 20% of the children had serologic evidence of a current or previous infection with the bacterium.

Bitar *et al*.[51] also failed to find any evidence of *H. pylori* by PCR in both middle ear fluid and adenoid tissue from children with chronic otitis media with effusion, despite positive results in the rapid urease test for some adenoid tissue samples.

Ozcan *et al.*[52] using immunohistochemistry and rapid urease testing, were also unable to find *H. pylori* in middle ear fluid and adenoid tissue, but 32 % of the children from which the samples derived had anti-*H. pylori* IgG antibody present in their blood, 12% had the anti-*H. pylori* IgA antibody, and 12% had both the anti-*H. pylori* IgG and the IgA antibody.

By contrast, Morinaka *et al*.[53] found that aspirated middle ear fluid samples from children with otitis media with effusion were almost all positive for *H. pylori* by immunohistochemistry and Giemsa staining. Bai *et al*.[54] used PCR and encountered 40% of middle ear fluid samples positive for *H. pylori* colonization in cases of otitis media with effusion. Karlidag *et al.*,[55] also using PCR, encountered only 16.3% of positive samples.

Fancy *et al.*,[56] on the other hand, when comparing adenoid tissue and middle ear fluid from children with chronic otitis media with effusion with adenoid tissue from a control group of children with indication for adenoidectomy but no middle ear pathology, detected *H. pylori* by PCR in the adenoids and middle ear fluid in a number of patients from both groups. However, the difference in *H. pylori*-positive adenoid samples between the study and the control groups was not statistically significant (10/45 and 6/37), which led them to conclude that, although *H. pylori* is definitely present in the adenoids (and

middle ear effusion) of some subjects in both groups, its presence does not support a role for the bacterium in the pathogenesis of otitis media with effusion.

Park *et al*.[57] showed that if 30% of the middle ear effusions tested positive for *H. pylori*, then *H. pylori* colonization at the adenoid tissue level in the same patients was not statistically different from that in controls.

Yilmaz *et al*.[58] performed biopsies of the middle ear promontorium mucosa, in parallel with the collection of adenotonsillar tissue samples, in two groups of pediatric patients, one with chronic otitis media with effusion and the other with no ear disease, and investigated the presence of *H. pylori* by PCR and culture. They encountered significantly higher rates of *H. pylori* colonization in both middle ear mucosa and adenotonsillar tissues in the otitis media group, which led them to conclude that the germ may have a role in the pathogenesis of otitis media with effusion.

Yilmaz *et al.*[59] encountered percentages of 47% of *H. pylori* colonization by PCR in middle ear fluid samples of children with otitis media with effusion, which constitutes evidence of a possible role for the germ in the pathogenesis of the disease, but did not find any *H. pylori* in the adenoid tissues, suggesting that adenoid tissue does not act as a reservoir for the bacterium.

Agirdir *et al*.[60] compared a group of children who presented middle ear fluid at myringotomy with a group of children who had no middle ear fluid when the tympanic membrane was incised, and observed that 66.6% of the collected middle ear effusions tested positive for *H. pylori* in the CLO test, but none of the wash-out liquid samples of children with no middle ear effusions tested positive for *H. pylori*.

Aycicek *et al.*,[61] using an animal model, concluded that *H. pylori* apparently does not play a role in the etiology of otitis media with effusion, but it may somehow contribute to the middle ear inflammatory process once other factors initiate it.

Kariya *et al*.,[62] also using an animal model, concluded the opposite: *H. pylori* induces a cascade of immunological and inflammatory changes in middle ear epithelium that suggests that the bacterium may play a significant role in otitis media with effusion pathogenesis.

Kutluhan *et al.*[63] investigated the possibility that *H. pylori* may also have a role in chronic suppurative otitis media, and collected middle ear tissue samples at tympano-mastoidectomy surgery and used PCR to observe that it is possible to detect the bacterium in the middle ear cleft and mastoid of 7.9% of chronic otitis media patients.

Dagli *et al.*,[64] also working in chronic suppurative otitis media, showed that 53.6% of the middle ear mucosa samples of patients undergoing tympano-mastoidectomy were positive for *H. pylori* in the CLO test, with those subjects scoring higher than controls in the urea breath test.

During one year, Saki *et al.*[65] had the middle ear mucosa of all of their chronic otitis media cases undergoing surgery assessed for *H. pylori* presence by PCR. They identified two groups of patients: those with tympanosclerosis, 84.2% of whom tested positive for *H. pylori*, and those without tympanosclerosis, 45.4% of whom were also *H. pylori*-positive. As the difference was statistically significant, and none of the other studied variables (including otorrhea and perforation size and location) correlated with *H. pylori* presence, the authors concluded that perhaps *H. pylori* has a role in the development of tympanosclerosis.

H. pylori and Sinusitis[2]

The hypothesis that *H. pylori* can have a role of in chronic rhinosinusitis etiopathogenesis was first introduced by Ozdek *et al*.[66] in 2003 after they detected the bacterium's DNA by PCR in the sinus mucosa of about 1/3 of patients undergoing sinus surgery. They proposed that the finding could either be the cause or a consequence of the pathology.

Almost simultaneously, Morinaka *et al*.,[67] using a combination of PCR, rapid urease (CLO) test, culture, and immunohistochemical analysis and defining positivity as at least two positive results in different tests, reported that two subjects (18%) in their series of patients with CRS who underwent sinus surgery had sinonasal tissue samples that tested positive for *H. pylori.* These two patients had, concurrently, either an evident or suspected *H. pylori* gastric infection.

Koc *et al*.[68] identified by immunohistochemical staining the presence of *H. pylori* in 20% of the surgical sinonasal samples of patients with nasal polyposis, whilst the samples collected during surgery for middle concha bullosa cases, which served as controls, were all negative for *H. pylori*. Both groups, however, displayed similar levels of IgG antibodies specific to *H. pylori* in their sera.

Cvorovic *et al*.[69] investigated the presence of *H. pylori* with a urease test and Giemsa staining simultaneously in the sinuses and the stomach of two groups of patients undergoing sinus surgery: one group had sinonasal polyposis and the other concha bullosa. They encountered *H. pylori* in 26% of the patients with polyposis, all of whom showed presence of the bacterium concomitantly in their stomach, whilst no patient with concha

[2] **Submitted** for publication to American Journal of Rhinology & Allergy

bullosa tested positive for *H. pylori.* They concluded that intranasal *H. pylori* is perhaps linked in some way to nasal polyposis, either as cause or as a consequence.

Kim *et al.*,[70] on the other hand, concluded that *H. pylori* probably does not play a critical role in CRS pathogenesis since only 25% of the patients in their series tested positive for *H. pylori* (using rapid urease testing and immunohistochemical analysis, with positive results confirmed by transmission electron microscopy), and that it was not possible to correlate severity of sinusitis (as assessed by both Lund-McKay CT-scan scores and symptom scores) with *H. pylori* intranasal colonization.

Ozyurt *et al*.[71] employed PCR to detect *H. pylori* and its major virulence factor, cagA, in nasal polyps, normal nasal mucosa and laryngeal tissue samples, coming to the conclusion that although the bacterium DNA was present in more than half of the specimens from the three sources (59.4 % of nasal polyps, 70.4 % of normal nasal mucosa samples, and 58.6 % of larynx samples), with cagA -positive strains dominating in all groups, there were no statistically significant differences between the normal mucosa group and the other two groups.

Burduk *et al.*,[72] in a group of patients who tested positive by PCR for *H. pylori* in sinonasal mucosa samples and benign laryngeal pathology samples, reported that cagA-positive *H. pylori* was identified only in laryngeal tissues and not in the sinus samples, leading to the conclusion that *H. pylori* may have a role in laryngeal pathology, but probably not in sinonasal disease.

Jelavic *et al.*[73] studied prospectively two groups of patients after sinus surgery: those with sinonasal samples positive for *H. pylori* and those with negative sinonasal samples, as evaluated by immunohistochemistry. They concluded that there was no difference

between the two groups in inflammatory status at surgery and postoperative improvement of subjective symptom scores, but surprisingly the *H. pylori*-negative patients performed poorly postoperatively on objective nasal endoscopic scores.

Včeva *et al*.[74] investigated the presence of *H. pylori* by PCR in sinonasal samples of two groups of patients, one with nasal polyposis and the other with concha bullosa, and found that *H. pylori* was present in the sinus mucosa of 28.5% of the polyposis cases whereas not a single concha bullosa case tested positive. *H. pylori* was indifferently present in the gastric mucosa of both groups, but *H. pylori* immunoglobulin G and A antibodies in the serum were found to be more common in polyposis than in concha bullosa patients (85.71% versus 53.33%), suggesting that *H. pylori* may have an active role in nasal polyposis pathogenesis.

Nemati *et al*.[75] employed three methods – PCR, culture, and urease test – to identify *H. pylori* in nasal specimens of two groups of patients, one with nasal polyps and the other with concha bullosa, deliberately excluding from the study any subject with evident symptoms of GERD. As no one in both groups tested positive, they concluded that there is no association between *H. pylori* and nasal polyposis in patients without GERD symptoms.

Nikakhlagh *et al.*[76] studied the presence of *H. pylori* DNA by PCR in the sinonasal mucosa of what is the largest studied population so far: 50 patients with CRS and 50 patients with concha bullosa. They found that 18% of the samples from CRS subjects tested positive for *H. pylori* DNA, while only 4 % were positive in the control group; this difference was statistically significant.

Cedeño *et al.*[77] studied the presence of *H. pylori* DNA by PCR in the maxillary sinus lavage fluid in a pediatric population with CRS, while simultaneously searching for the bacterium, using histology techniques, in the children's adenoid tissue and for specific secretory immunoglobulin A against *H. pylori* in the saliva. They observed that none of the children with CRS had evidence of the germ's presence, neither in their maxillary sinuses nor in their adenoids, in spite of 28.6% of them showing previous immunogenic contact with *H. pylori* in their saliva.

In another recent study, Bansal *et al.*[78] concluded that *H. pylori* seems to be associated with nasal polyposis, since the pathological sinus mucosa of 35 patients with nasal polyps showed much higher detection rates of *H. pylori*, by histology and immunohistochemistry, when compared to the normal nasal mucosa from 35 septoplasty subjects, which served as controls (40% versus 8.5%). Furthermore, two histopathological features in the nasal polyps – hyperplasia of the pseudostratified ciliated epithelium and aggregates of infiltrating lymphocytes – were statistically associated with local presence of *H. pylori* in the mucosa. Patients and controls also underwent stool antigen testing in the same study, which showed that all subjects testing positive for intranasal *H. pylori* had simultaneously positive stool antigen tests.

The previously mentioned meta-analysis study on reflux and CRS by Leason *et al.*[26] also presented data on *H. pylori* in sinonasal tissues from all the 10 case-control studies published until January 2015. Assessing, using various methodologies, sinonasal *H. pylori* presence in 265 subjects with CRS in comparison to 188 healthy individuals, the meta-analysis found an increased odds ratio (OR) of *H. pylori* in CRS (OR 2.88) overall, while *H. pylori* prevalence in CRS was found to be 31.7%. The analysis of studies when

both intranasal *H. pylori* and GERD were evaluated revealed that 87.5% of subjects with intranasal *H. pylori* also had reflux.

HYPOTHESIS AND GOALS OF THE INVESTIGATION

The data generated so far supports for and against arguments for an eventual role for LPR and *H. pylori* in inflammatory diseases of the upper aerodigestive and respiratory tract, including CRS.

The reported clinical improvement seen in a number of cases after anti-reflux or anti- *H. pylori* therapy appears to be a rather convincing, albeit totally empirical argument, but the documentation of LPR and *H. pylori* in the aerodigestive and respiratory tract seems to be still plagued by many unresolved methodological issues and does not necessarily establish a causal link to airway inflammatory disease.

Definitely, a final word on the subject has not been said yet, and the current data are closer to establish that an association between both LPR and *H. pylori* and CRS does in fact exist, than to enlighten us on the nature of that association.

We test here the hypothesis that LPR and its contents, including *H. pylori*, are in some way associated with chronic inflammatory disease of the upper respiratory tract, and we chose chronic inflammatory disease of the paranasal sinuses as the target disease model for the investigation.

The objectives are:

1) To investigate the relative role of LPR contents, *H. pylori*, pepsin and pepsinogen I in the development of sinonasal inflammation in a case-control study of patients selected for sinus surgery due to chronic medically refractory rhinosinusitis. The results obtained from patients with chronic rhinosinusitis are to be compared to the results of individuals with concha bullosa, serving as controls.
2) To investigate the relative role of *H. pylori* in the development of sinonasal inflammation, comparing the bacterium's presence in the nose with its gastric colonization pattern, in a cohort study of patients selected for sinus surgery due to chronic medically refractory rhinosinusitis.

CHAPTER TWO

HELICOBACTER PYLORI AND LARYNGOPHARYNGEAL REFLUX IN CHRONIC RHINOSINUSITIS [3]

[3] **Published** as Dinis PB, Subtil J. Helicobacter pylori and Laryngopharyngeal Reflux in Chronic Rhinosinusitis. *Otolaryngol Head Neck Surg* 2006; 134: 67-72.

OBJECTIVE: Investigation of the potential role of several laryngopharyngeal reflux contents in sinus disease.

STUDY DESIGN AND SETTING: A controlled cohort analysis of *Helicobacter pylori*, pepsin and pepsinogen I in inflamed and non-inflamed sinonasal tissue. Fifteen patients, selected for surgery due to chronic medically refractory rhinosinusitis, had their pathologic sinus tissue analyzed for polymerase chain reaction detection of *H. pylori* DNA and assayed for pepsin and pepsinogen I tissue concentration levels. A control group of 5 subjects undergoing surgery for anatomic sinonasal abnormalities provided non-inflammatory mucosa specimens for comparison.

RESULTS: *H. pylori* was found scattered in inflamed and non-inflamed mucosa, whereas sinonasal tissue pepsin/ pepsinogen never rose above blood levels in both groups.

CONCLUSIONS: Evidence of intra-operative peptic reflux into the sinuses was not found. As *H. pylori* was similarly encountered in healthy and diseased sinus mucosa, it seemingly fails to support a pathogenic role for this organism in the sinuses.

INTRODUCTION

Gastric reflux has been incriminated recently in a myriad of laryngeal and other supra esophageal symptoms,[1,2] a much debated topic under the banner name of laryngopharyngeal reflux (LPR) disease. Patients with clinical LPR undergoing pH monitoring have documented acid reflux intermittently present in their pharynx, which can sometimes be traced as high in the airway tract as the nasopharynx,[3-5] with gastric pepsin being detected even in middle ear effusion of children with secretory otitis media.[6] Due to the protean nature of LPR symptoms, a host of controversies remain.[2] A hypothetical relation between LPR and chronic inflammation of the nose and sinuses was further added to the debate, in acknowledgment of the fact that both conditions intersect fairly consistently in clinical practice.[7-9] Reports of improvement of chronic sinusitis in children after anti-reflux therapy apparently support this relationship,[4,10,11] whereas omeprazole is able to reputably improve symptoms of refractory chronic sinusitis in adults.[12]

A number of pathophysiologic mechanisms for LPR symptoms have been proposed. Because *Helicobacter pylori* is prevalent in gastric contents, this organism has also been added to the list of potential role players in reflux-induced supra esophageal mucosa injury. *H. pylori* was declared a likely laryngeal pathogen[13] and its presence was also detected in diseased sinonasal tissue.[14,15] Thus far, however, no well designed controlled studies have emerged to clarify the role of this organism in upper respiratory tract inflammation.

MATERIALS AND METHODS

Fifteen, 11 male and 4 female, adult patients, aged 18 to 79 (mean 49.9 years), requiring endoscopic sinus surgery due to medically recalcitrant chronic rhinosinusitis involving the anterior and the posterior ethmoid on at least one nasal cavity, with or without simultaneous sphenoid sinus disease, were enrolled as a study group (Figure 1). Informed consent was obtained from each patient and the study protocol was subject to approval by the institutional ethics committee. All subjects had their biochemical and hematological profiles within the normal range, with the exception of mild to moderate eosinophilia found in some atopic patients. Patients with cystic fibrosis, immotile cilia syndrome and known immunodeficiencies were excluded. In all subjects the inflammatory nature of the disease was confirmed subsequently by hystopathologic examination.

Five, 1 male and 4 female, adult patients, aged 22 to 72 (mean 37.6 years), requiring endoscopic sinus surgery due to endonasal anatomic variations (symptomatic concha bullosa) that did not produce CT scan detectable sinus inflammation, were enrolled as a control group (Figure 2).

In the immediate pre-operative period of all subjects, drug therapy for any underlying condition was allowed, including systemic steroids for asthma management. Excluded from the investigation, however, were patients who undertook antimicrobial therapy of any kind, including in prophylaxis, in the last weeks before surgery. Also, no patient had undergone treatment with antacids, histamine$_2$ -receptor antagonists, prokinetic agents, or proton-pump inhibitor in the recent pre-operative period. No patient had a history of prior fundoplication or Barrett's esophagus.

Surgery was performed under general anesthesia, with orotracheal intubation. No patient required nasogastric tube placement and vomiting did not occur, pre- or intra-operatively, according to anesthesia chart review.

In both groups, blood samples (7ml) were collected immediately before induction of general anesthesia. Serum was separated by centrifugation and immediately frozen at –70ºC until assayed. Concentrations of pepsin and pepsinogen I were subsequently determined, the former by an immunoassay with rooster polyclonal antibodies to purified human pepsin, [16] the latter by radioimmunoassay.[17]

Preceding the intervention, routine vasoconstriction of the nasal mucosa to minimize intra-operative bleeding, was carried out, including infiltration, at critical sites, with 1% xylocaine with 1:100,000 epinephrine.

During surgery tissue samples of sinonasal mucosa were collected in the study group at the anterior ethmoid (including material taken from the uncinate process, ethmoidal bulla and meatal portion of the middle turbinate), the posterior ethmoid; and, eventually, C) the sphenoid sinus. The extent of the pathology on the CT scans determined which sites were to be surgically addressed, but pathology at anterior and posterior ethmoid on at least one side of the nose, with or without associated sphenoid disease, was required for study enrollment (Figure 1).

In the control group, tissue samples were collected both from the meatal portion of the middle turbinate of the concha bullosa, which served as anterior ethmoid specimen, and from a donor site of healthy, posterior ethmoid, mucosa on the same side of the nose (Figure 2). The latter specimens were collected through a small opening at the basal lamella, with the utmost care to minimize collateral damage to nasal anatomy and

function. One or both sides of the nose were addressed this way according to pre-determined require-to-treat surgical planning. Site inflammation was ruled out subsequently by hystopathologic examination, whereas post-operative endoscopic control warranted that proper healing occurred in all operated sides.

In both groups, the mucosa specimens collected at all the different sinonasal sites were immediately frozen at –70ºC and assayed subsequently for 1) pepsin and pepsinogen I concentration levels, using an immunoassay with rooster polyclonal antibodies to purified human pepsin[16] and radioimmunoassay methodology,[17] and for 2) polymerase chain reaction (PCR) extraction and amplification of genomic *H. pylori* DNA.[18] The method, described elsewhere,[17] employed the PCR – based microtiter plate hybridization technique with primers targeted to an *ureA* gene segment of *H. pylori*. The sensitivity of the method is 10 bacterial cells, with 100% specificity. Statistical analysis employed the following tests according to specific requirements: the Mann-Whitney test, Wilcoxon Signed-Rank test, paired-samples *t* test, Pearson's chi-square test, Fisher's test and the McNemar test. For all, a significance level was set at $p = .05$.

RESULTS

Control group patients are mostly female and younger in age and study group patients seem biased toward the male gender and have higher rates of allergy, asthma and aspirin intolerance co-morbidity. However any statistic analysis comparisons of the demographics of both groups are affected by the small number of patients in the control group.

Figure 3 illustrates the study group mean concentration results and standard deviations of pepsin and pepsinogen I in serum and in sinonasal tissue. The tissue/ blood ratio for both pepsin and pepsinogen I was 0.17. No statistically significant differences were found between patients and controls regarding blood and mucosa pepsin and pepsinogen I values. Sex, site, nasal side, disease extension, and co-presence of allergy, asthma and aspirin intolerance, were not found to have an influence on the sinus tissue concentration levels of both enzymes. Age, however, was found to influence, as older patients tend to have higher sinonasal concentrations of both pepsin (p= .007) and pepsinogen I (p= .03).

Table I displays the results of *H. pylori* distribution per patient and surgical site in all subjects. From the 69 tissue samples collected in the study group 19% were positive for *H. pylori* infection, whilst from the 12 tissue samples collected in the control group 8% were *H. pylori* positive. No statistically significant differences were encountered in *H. pylori* colonization between diseased mucosa and control sinonasal tissue. Also, in both groups, no variable, such as age, sex, nasal side, site, disease extension, co-presence of allergy, asthma or aspirin intolerance, was found to relate to bacterial colonization. Even concomitant pepsin and pepsinogen I tissue levels were not found to statistically relate to *H. pylori* presence in any specific site, in both groups.

DISCUSSION

Our study shows that the concentrations of pepsin and pepsinogen I in the sinuses are in fact no higher than the pepsin and pepsinogen serum levels, with no single mucosal result ever exceeding blood concentrations. As the extracellular fluid in chronically inflamed sinonasal mucosa results from plasma transudation processes, we needed to clarify if the pepsin levels detected at the mucosa were the result of pepsin from gastric juice or,

simply, pepsin derived from plasma pepsinogen. The latter seems to be the case, as pepsin tissue levels are compatible with plasma transudation of pepsinogen into the sinus mucosa rather than a gastric reflux transport of the enzyme into the nose, in which case values up to 1000-fold the serum levels can be expected.[6] This finding does not preclude the possibility that gastric contents may reflux into the nasal cavity in LPR patients. Merely that no evidence of refluxate reaching the nose and sinuses could be documented during the time these patients were in surgery under general anesthesia. A conclusion that perhaps should not surprise since LPR patients are typically upright position daytime refluxers,[2] and as few as three reflux episodes per week are apparently sufficient to produce ongoing inflammation at the highly sensitive supra esophageal mucosa.[1,2]

Our study also reveals that *H. pylori* is present in the sinonasal mucosa of a significant number of chronic rhinosinusitis surgical specimens. These results are in agreement with data published previously,[14,15] the occurrence perhaps being even more prevalent than reported.[14] The fact that, in our series, pre-operative antimicrobial prophylaxis was avoided and no patient was taking H_2 -receptor antagonists or proton- pump inhibitors prior to surgery, may have enhanced the diagnosis sensitivity. The study design also allowed for a mapping distribution of the organism within the sinonasal cavity, which, however, did not reveal any particular pattern of colonization. Therefore the posterior sinuses (i.e., posterior ethmoid and sphenoid sinus) are at no greater risk of being colonized by the organism than the anterior ethmoid, as gastric backflow mechanism would have it. Unless unforeseen factors have biased our data (i.e., lidocaine has been shown to inhibit the *in vitro* growth of the organism), nasal colonization by *H. pylori* in

chronic rhinosinusitis has a patchy distribution and does not seem to follow an established pattern.

The pathophysiology of LPR disease is a much debated topic, with the mechanisms direct peptic-acid injury and triggered neurophysiological changes, cited most frequently.[2,9] The possibility that *Helicobacter pylori* may also play an etiopathogenic role was only put forth very recently.[14,15] This organism is prevalent in the gastric contents of a large number of humans worldwide.[19] Once infected, adults will maintain lifelong *H. pylori* colonization, until eradication by specific therapy.[19] Gastric infection is characterized by a non-invasive mucosal surface attachment of the bacterium that triggers a specific host response, involving neutrophils, T and B lymphocytes, plasma cells, and macrophages, ultimately leading to tissue damage.[19] Although expressed clinically in highly variable ways, virtually all *H. pylori* -infected persons have chronic superficial gastritis, often displaying a patchy distribution at the stomach mucosal surface[19] (not unlike the colonization pattern found in the sinuses).

Since a significant number of gastroesophageal reflux disease (GERD) patients are infected with *H. pylori*, and the infection seems to disturb lower oesophageal sphincter function,[19] the hypothesis of a refluxate-conveyed bacterial seeding of the supraesophageal mucosa in LPR patients is perhaps not too far-fetched. It has been suggested that the reflux of infected gastric juice into the nasopharynx may produce airway mucosal edema and inflammation from the combined activity of the peptic–acid injury *and* local *H. pylori* infection.[14] A direct relation between the severity of the mucosal inflammation and sinus *H. pylori* colonization rates can even be argued for, since sinus surgical failure patients, with surgically as well as medically recalcitrant

pathology, as opposed to medical-only failures from our studied population, thus apparently with more severe sinus disease, have been shown to suffer from an increased prevalence of LPR.[5] The < 100%-positive bacterial identification rate scenario we have identified in the inflamed sinus mucosa could also be mimicking what is reported to occur at other colonization sites in the body: that repeated and extensive sampling is frequently required to ensure that a certain diagnostic methodology does not miss out on the *H. pylori* mucosal infection. And if the T helper (Th) 1-associated *H. pylori*-inflammation at the stomach[19] seems at odds with the Th2 eosinophilic pattern that histopathologically hallmarks chronic rhinosinusitis at the nose, one should acknowledge possible similarities between the latter and an entity called eosinophilic esophagitis. [20] The latter often mimics GERD but is characterized by a lack of response to acid suppression,[20] a feature shared with severe LPR.

Any relation, however, remains speculative. At no other location than the gastric mucosa was *H. pylori* found to cause disease, although the organism was also detected in saliva, dental plaque, adenoids and tonsils.[19] The presence of the bacterium in the sinuses may even be the result of a direct transmission of the infection from any of these sites into the nasal cavity. If the role of *H. pylori* in extragastric locations is presently unclear, some believe they serve as reservoir sites for gastric re-infection, as recurrence after antimicrobial therapy is not uncommon.[19] The fact that in our study, *H. pylori* colonization was not statistically found to be more common in sinusitis patients than in controls, lends credibility to the *nose as a reservoir* thesis. Whether the nose acts as a permanent or a transient extra-gastric settling place for the organism is not known, the latter hypothesis agreeing more with the pattern of inconstant identification of the

organism in sinonasal mucosa we have found. Thus, potential clinical benefits of anti-reflux therapy in chronic rhinosinusitis[4,10-12] seems more likely to result from the suppression of the LPR-induced, peptic-acid or neurologic, pathogenic effects on the upper airway mucosa, rather than from an influence on the nose and sinuses' *H. pylori* colonization. Although we acknowledge that our studied population is perhaps too small in size to allow a definitive word on this matter, our conclusions are nevertheless remarkably consistent with the recent concept that, when in co-morbidity, the pathogenic mechanisms of *H. pylori* infection and reflux disease probably run in a parallel fashion, independent from each other.[19]

CONCLUSION

If the role of *H. pylori* infection in gastroduodenal disease is unquestionable, the manner by which the organism is transmitted remains unclear. To the established fecal-oral and oral-oral routes, newer routes of transmission have been proposed such as gastric-oral and, more recently, gastric-nasal transmission, the latter the aim of our investigation. *H. pylori* DNA may be encountered in chronically inflamed sinonasal mucosa, possibly as a manifestation of LPR disease, although at this time we can only speculate on the bacterium's doings at this particular location. The possibility that it plays some etiopathogenic role in rhinosinusitis can not be dismissed too lightly, as theoretically there is some valid reasoning behind it. Our data, however, does not seem to substantiate a pathogenic presence of the organism in the nose. At the sinonasal mucosa *H. pylori* more likely has a reservoir function similar to other extragastric, upper aerodigestive, site infections, possibly contributing to the oral-oral route of transmission or instigating gastric re-infection.

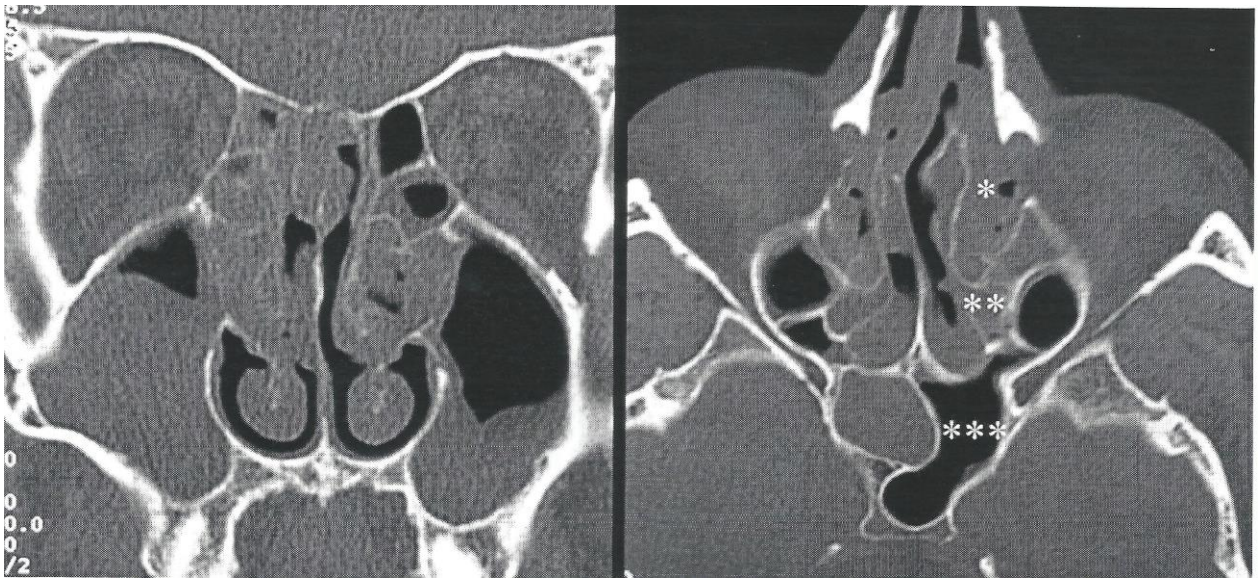

Figure 1. CT-scan of a typical study group patient, with extensive chronic inflammatory sinus disease requiring surgery for bilateral anterior (* left hand-side on the axial plane) and posterior ethmoid (**) sinuses pathology, with sphenoid sinusitis on the right hand-side. A total right sphenoethmoidectomy with total left ethmoidectomy was performed, which provided diseased tissue samples from the right and left anterior ethmoids, right and left posterior ethmoids, as well as right sphenoid sinus. The left sphenoid sinus (***), spared surgically, failed to contribute a site sample to the investigation.

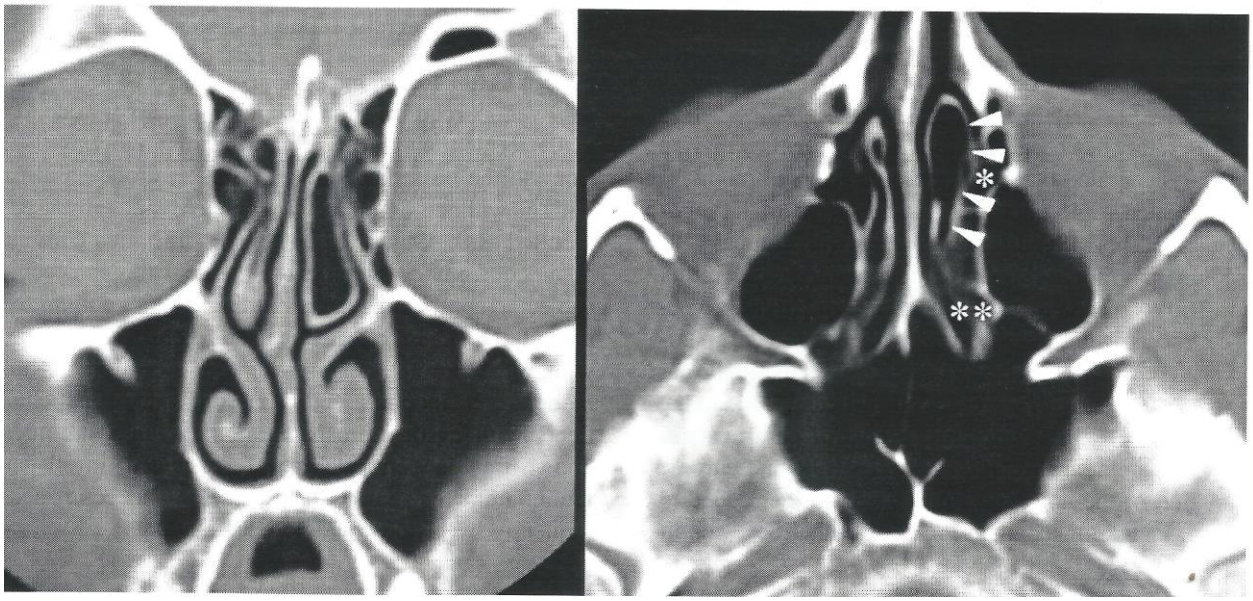

Figure 2. CT-scan of a typical control group patient, requiring surgery for a symptomatic left concha bullosa (* axial plane), but without imaging evidence of inflammation in any part of the sinuses. The meatal mucosa of the partially ressected middle turbinate, signaled by the arrows, was labeled non-inflamed anterior ethmoid sample, whereas a posterior ethmoid non-inflamed mucosa sample from the same side (**) was collected through a small opening at the basal lamella. The right side of the nose was not surgically manipulated, thus failing to contribute any site sample to the investigation.

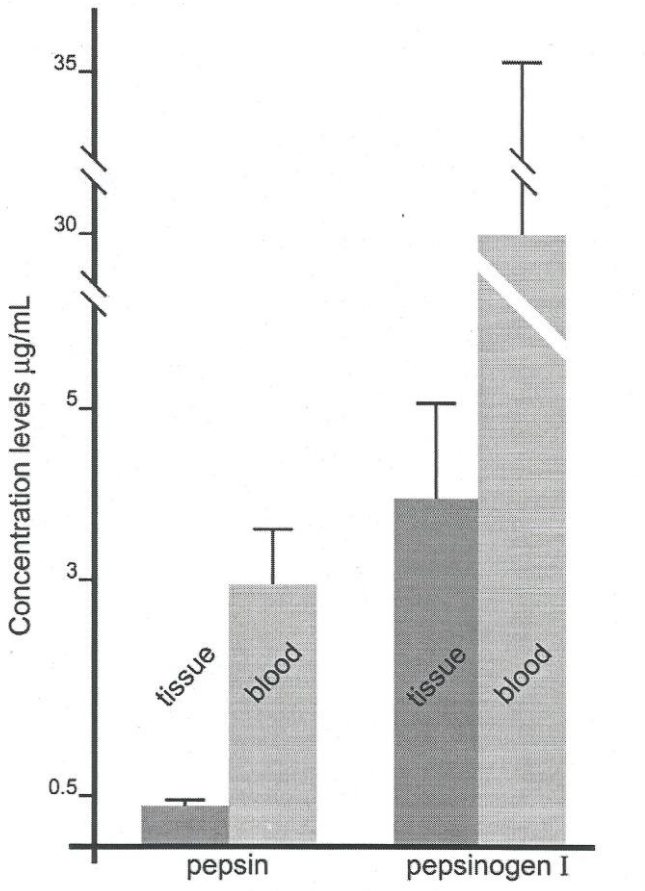

Figure 3. Mean concentration levels and standard deviations of pepsin and pepsinogen I in sinonasal tissue and serum in the study group.

CHAPTER THREE

HELICOBACTER PYLORI

IN BOTH THE SINUSES AND THE STOMACH[4]

[4] **Published as** Dinis PB, Matos T, Sardinha M, Alves PL, Vital J, Carvalho AM, Vitor J. Helicobacter pylori In Both the Sinuses and the Stomach. *Rhinology Online* 1: 194-200, 2018.

Background: The role played by *Helicobacter pylori* in the sinuses, and its association with the same organism's gastric infection, are still unclear.

Methods: In order to compare *H.pylori* colonization patterns in the nose and stomach we conducted a cohort analysis of 14 patients, eligible for sinus surgery due to chronic medically refractory rhinosinusitis, who were tested for simultaneous presence of *H. pylori*, by histology, culture and polymerase chain reaction, in pathologic sinus tissue collected during surgery and in gastric mucosa obtained through gastroduodenal endoscopy.

Results: *H. pylori* DNA was found in the sinus mucosa of 15.4% of patients with chronic rhinosinusitis, and all of them showed concurrent *H. pylori* stomach infection. Sinus colonization was not found without simultaneous gastric colonization, although most patients with gastric infection did not have the bacterial DNA in their sinuses. *H. pylori*'s presence in the nose was not associated with local inflammatory status, and no cultures could be obtained from any of the sinus tissue samples, including those positive for *H. pylori* DNA.

Conclusions: Only *H. pylori* DNA, and not the culturable active form of the microorganism, could be found in the sinus mucosa of some patients with *H. pylori* gastric infection. We could not find evidence, however, that the bacterium's presence in the nose contributes to local mucosal inflammation.

INTRODUCTION

Helicobacter pylori DNA has been detected in a number of extra-gastric locations, such as the oral cavity, tonsils and adenoids,[1,2] and even the middle ear and paranasal sinuses.[2-15] However, the significance of the microorganism's presence at these aero- digestive and respiratory sites is still unclear. It is believed that the oral cavity is an important reservoir for *H. pylori,* that contributes to the oral-oral route of transmission and acts as a source of stomach re-infection.[1] Others suggest that the bacterium may be capable of causing damage to the aero-digestive and respiratory mucosa in the same way it does to the gastric mucosa.[1] However, this capacity for extra-gastric disease remains unproved, and some authors argue that it even seems unlikely. In order to initiate tissue damage, not only does *H.pylori* require a set of events unique to the gastric milieu, but also culturable active forms of *H. pylori* have only been recovered outside the stomach in the aero-digestive tract in the vicinity of the upper esophagus (i.e. in tracheal secretions in intubated patients), and never as far away as the bronchi, lung, or the upper respiratory tract.[2] That far from the stomach, only the microorganism's DNA suggests the presence of *H. pylori* at such locations.

We conducted the present investigation in order to see how *H. pylori* colonization patterns in the nose and stomach are associated in chronic rhinosinusitis patients, as a way to also provide information on the eventual existence of an interaction between two seemingly unconnected pathologies, one in the digestive tract and the other in the upper respiratory tract. At the same time, we assessed if this known gastric pathogen has the capacity to also inflict direct injury to the sinus mucosa.

MATERIALS AND METHODS

A total of 14 adult patients, 8 male and 6 female, aged 30 to 68 years (mean 48.1 years), requiring endoscopic sinus surgery due to medically recalcitrant chronic rhinosinusitis, with and without nasal polyposis, were enrolled in a consecutive, unselected manner. Informed consent was obtained from each patient, and the study protocol was approved by the institutional ethics committee. All patients had their biochemical and hematological profiles within the normal range, with the exception of mild-to-moderate eosinophilia in some atopic patients. Patients with cystic fibrosis, immotile cilia syndrome, and known immunodeficiencies were excluded. The inflammatory nature of the disease was subsequently confirmed by histopathologic examination in all patients enrolled.

Drug therapy for any underlying condition was allowed, including systemic steroids for asthma management. Antibiotic use in the 3 months prior to surgery was also noted. Moreover, patients were specifically asked about their history of prior *H. pylori* infection diagnosis and treatment, as well as current medication with antacids, histamine 2 - receptor antagonists, prokinetic agents, or proton-pump inhibitors (PPI's).

In the immediate pre-operative period (from 1 to 10 days pre-op), all subjects underwent a ^{13}C-labelled urea breath test.

Surgery was performed under general anesthesia, with orotracheal intubation. Routine vasoconstriction of the nasal mucosa was carried out to minimize intra-operative bleeding, including infiltration, at critical sites, with 1% xylocaine with 1:100,000 epinephrine.

The extent of the pathology on the computed tomography scans determined which sites were to be surgically approached. All patients required bilateral procedures due to

roughly symmetrical extension of the pathology, according to the Lund-McKay classification.[16] During surgery, tissue samples of diseased sinonasal mucosa were collected at the anterior ethmoid (including the uncinate process, ethmoidal bulla, and meatal portion of the middle turbinate), and the posterior ethmoid sinus from one, randomly chosen, nasal cavity. The collected sinus specimens were subjected to: 1) histopathological examination, to assess the overall degree of local inflammation (graded as 0+, no inflammation; 1+, mild; 2+, moderate; and 3+, severe inflammation, depending on the inflammatory infiltrate cell density)[17]; 2) microbiological culture, to isolate *H. pylori;* and 3) polymerase chain reaction (PCR) to amplify genomic *H. pylori* DNA.

Enrolment required that the patients also agree to undergo a simultaneous upper gastrointestinal endoscopy, irrespectively of digestive symptoms. Biopsies were taken from the stomach mucosa lining, either from endoscopic-assessed suspected sites or iteratively, from the lesser or greater curvature of the antrum and the greater curvature of the corpus. Patients underwent gastroduodenal endoscopy either simultaneously with the sinonasal mucosa sampling (intra-operatively, under general anesthesia, while waiting for the vasoconstriction effect to occur in the nose) or up to two weeks post sinus surgery. The collected gastric biopsy specimens from the two sites, the antrum and the corpus, were analyzed by: 1) histopathology, to assess the overall degree of local inflammation (graded as 0+, absent; 1+, mild; 2+, moderate; 3+, severe inflammation, depending on the inflammatory infiltrate cell density); 2) histology to identify *H. pylori* in Warthin-Starry silver stained gastric mucosal biopsies, (also graded as 0+, no bacteria found; 1+, mild ; 2+, moderate; 3+, severe density colonization); and 3) microbiological culture of gastric biopsy specimens specific for *H. pylori*.

All sinus and stomach tissue samples were collected aseptically, transported to the laboratory at 4ºC in Portagerm pylori (bioMérieux, France), and processed less than four hours after collection. For microbiological culture, a drop of tissue macerate was plated onto *H. pylori*-selective medium (Brucella supplemented with 10% horse blood and Brucella supplemented with 10% horse blood and Oxoid *H. pylori*-selective supplement (Dent)) and incubated at 37ºC in a microaerophilic atmosphere (Campygen, Oxoid) for up to 15 days. Colonies were then tested for urease, catalase and oxidase, and motility and were stained with Gram stain.

For PCR testing, two sets of primers targeting the 16S rRNA and 23S rRNA genes of *H. pylori* were used, chosen to maximize detection as together they target sequences common to virtually all *H. pylori* strains.[18,19] The first primer pair, PCR-G, amplifies a 780bp fragment from the 16S rRNA gene specific for the *Helicobacter* genus, Helico F – 5' CTATGACGGGTATCCGGC 3' and Helico R – 5' CTCACGACACGAGCTGAC 3'. [(18)] The second primer pair, PCR-S, amplifies a 267 bp fragment from the 23S rRNA gene of the *H. pylori*, HPYS - 5´ CGCATGATATTCCCATTAGCAGT 3' and HPYA - 5' AGGTTAAGAGGATGCGTCAGTC 3'.[19] Ethidium bromide-stained agarose gel electrophoresis was employed for separating the PCR products according to their size. When interpreting the results, we considered a positive genus-specific PCR test a sign of presence of DNA for one of the various *Helicobacter* species, whilst the combination of a positive genus-specific with a positive species-specific PCR test was taken as a clear sign of presence of the actual *H. pylori* species.

For histological identification of *H. pylori* in paraffin sections of sinus tissue, the modified Warthin-Starry silver staining kit (Merck, Germany) was used.

The grading of the histological material, as well as all the molecular biology procedures, were performed after the identification of the samples was masked.

Statistical analysis employed descriptive statistics and the Spearman's rank correlation coefficient (Spearman's rho).

RESULTS

The study group included 8 patients with asthma, 6 with allergic rhinitis, and 2 with intolerance to nonsteroidal anti-inflammatory drugs (NSAIDs). Other co-morbidities included: hypertension (n=2), hypothyroidism (n=1), goiter (n=1), angioma of the liver (n=1), ankylosing spondylitis (n=1), fibromyalgia while on NSAID's (n=1), gastroesophageal reflux disease (GERD) (n=2) and chronic gastritis (n=1). Two patients were undergoing revision sinus surgery, and three admitted having taken an oral antibiotic in the immediate three months prior to surgery. One subject had history of *H. pylori* gastric infection treatment, while five were taking a PPI until the day before surgery. The patient with ankylosing spondylitis was under treatment with sulfasalazine at the time of the surgical procedure, and the stiffness of his entire spine was so severe that the gastroenterologist was unable to perform the required gastroduodenal endoscopy, even under general anesthesia. Another patient underwent upper gastrointestinal endoscopy outside of the time frame imposed by the strict study criteria (either intra-op or up to two weeks post sinus surgery), so the gastric results were excluded from the analysis. Two patients had histology results for one gastric site only, and in one patient the gastric histological results were altogether absent.

The inflammatory status of the sinus mucosa at the time of surgery for each patient is displayed in Table 1. Severe inflammation was not encountered in any subject; moderate

inflammation was found in 50% (n=5) of the asthmatics and in 16.6 % (n=1) of the non-asthmatics, whilst mild inflammation was encountered in 50% (n=4) of the asthmatics and in 83.3 % (n=5) of the non-asthmatics. A total of 60% (n=3) of patients in the moderate sinus inflammation group had allergic rhinitis, while mild inflammation was observed in 50% (n=3) of the patients with a diagnosis of allergic rhinitis. All patients with intolerance to NSAIDs in the study group displayed moderate sinus inflammation.

Moderate sinus inflammation was also encountered in 66.7% (n=2) of the patients who had taken an antimicrobial agent in the three months prior to surgery, whilst mild inflammation was mostly found (72.7%, n=8) in patients who had not recently needed antibiotic treatment.

The only patient who had prior treatment for *H. pylori* gastric infection displayed moderate sinus inflammation, while, among the five patients who were on PPI's at the time of surgery, three showed mild sinus inflammation and two moderate sinus inflammation.

Table 1 shows the results of the gastric and nasal inflammatory and *H. pylori* infection status for all patients.

All patients were found to have some degree of gastric inflammation, fitting the histopathological diagnosis of chronic non-atrophic gastritis (active or non-active), even when the endoscopist failed to identify inflammation and performed iterative biopsies. A total of 60% of the antrum biopsies showed moderate inflammation, with mild inflammation occurring in 40%, whereas in corpus biopsies 70% had mild and 30% moderate inflammation.

Histological identification of *H.pylori* in gastric mucosa biopsies of the antrum was negative in 50% of the cases (n=5), revealing mild infection in 10% (n=1), moderate infection in 10% (n=1), and severe infection in 30% of the cases (n=3). For the corpus biopsies, the results were negative in 50% (n=5), with mild infection in 10% (n=1), moderate in 30% (n=3), and severe infection in 10% (n=1).

The results of cultures of gastric mucosa biopsies were positive for *H.pylori* in 66.7% of the cases (n=8), whilst the PCR-G test was positive in 54.5% (n=6) of the antrum samples and in 50% (n=5) of the corpus samples, and the PCR-S test was positive in 72.7% (n=8) of the antrum samples and in 66.7% (n=8) of the corpus samples.

A total of 66.6% of the patients receiving PPI treatment were *H. pylori* negative.

Table 2 shows the correlations between the variables found to have statistical significance.

Regarding *H.pylori* presence in the sinonasal mucosa, the species-specific PCR identification was positive in only two cases (15.4%), although 69.2% had sinonasal samples with positive genus-specific PCR results. The first case of positive *H.pylori* DNA in the sinuses had a positive breath urea test and tested positive for *H.pylori* in the stomach by histology, bacterial culture and PCR. The second case, however, had a negative breath test and tested negative for gastric *H.pylori* by histology and bacterial culture, but the species-specific PCR identification test in the stomach was positive (result confirmed at a different laboratory).

All attempts to grow *H.pylori* in culture medium from sinonasal samples failed.

DISCUSSION

The characteristics of our investigation, with patients undergoing simultaneous extensive sinus and stomach tissue sampling, and with various tests concurrently performed,

necessarily restricts the number of patients enrolled in the study, and as a result may not be able to show statistical significance. The available data, however, are sufficient to allow for important conclusions.

Since urease production is a hallmark of active gastric infection, it is perhaps no surprise that most, but not all, patients in our study with a positive urease breath test had indeed *H. pylori* gastric infection, confirmed by histopathology, culture, and/or PCR testing. The breath test was positive in one case where *H. pylori* was detected in the sinuses, but was negative in the other. As the majority of patients in our study had positive breath tests and no evidence of *H. pylori* in their sinuses, this apparently renders the test unsuitable for the specific identification of the bacterium in the nose.

Our data actually reveals that, in spite of the relevant number of patients with positive *Helicobacter*-genus DNA detected by PCR in the sinus mucosa (which certainly merits separate investigation), specifically *H. pylori* DNA was only found in the sinonasal mucosa of about 15.4% of chronic rhinosinusitis surgical patients. These results are in agreement with previously published data.[3-15] Admittedly, this prevalence could indeed be greater if the bacterium in the nose would follow a similar mosaic pattern of mucosal infection as in the stomach, with patches of diseased mucosa alternating with non-infected normal mucosa, in which case it would require repeated and extensive sampling to allow for a positive *H. pylori* result (five different sites, according to some).[20]

Both cases with *H. pylori* DNA in the sinuses had *H. pylori* DNA simultaneously present in their stomachs, but only about 25-28.6% of the patients with positive gastric species-specific PCR tests had *H. pylori* DNA in their sinuses. This suggests that if the bacterium is to be encountered in the nose, its DNA has to also be present in the stomach, but that

not all *H. pylori* gastric infections are necessarily associated with *H. pylori* colonization of the sinuses.

Our data show that while there is a positive correlation between cultural and histological identification of *H. pylori* in the stomach and gastric inflammation, we found no such correlation between *H. pylori* and site inflammation in the nose (Table 2). Therefore, it is perhaps not farfetched to admit that *H. pylori* presence in the nose and sinuses does not contribute to local mucosal inflammation.

The co-diagnosis of allergic rhinitis, asthma or intolerance to NSAIDs, was not found to statistically relate to either positive or negative sinonasal *H. pylori* results, suggesting that sinonasal *H. pylori* colonization may occur regardless these co-morbidities are present or not.

Critically, *H. pylori* could only be recovered from the nose in the DNA form, as all the attempts to culture the bacterium from nasosinusal sites failed. This inability to culture *H. pylori* could be due to the presence of too few microorganisms to be detected, or the simultaneous presence of too many types of other bacteria in the nose that inhibit growth of *H. pylori*. However, it has been shown that the bacterium can be cultured from adverse environments such as the air sampled during vomiting or from a tracheostomy tube.[2] So it is admissible that the reason may not have to do with the method but with the possibility that, in the sinuses, either the organism is represented just by fragments of its DNA and that these are destined to transiently remain there just for a limited time, or the microorganism is, in fact, in a dormant state that precludes culture, and is destined to remain for a long time in the sinuses.

The bacterium is, indeed, known to be able to resist harsh environments by changing to a dormant, inactive state, a non-culturable coccoid form that could still be potentially viable, later on, in the stomach.[21,22] It is therefore possible that *H. pylori* may lay dormant for long periods of time, using the nose and the sinuses as reservoirs, waiting for an eventual return to an active form, either to cause gastric re-infection or to participate in the oral-oral route of transmission. The fact that in a previous study no statistical difference was observed between *H. pylori* nasal colonization in patients with sinusitis when compared to the control group,[6] lends credibility to the '*nose as a reservoir'* thesis, and reinforces our conviction that the bacterium's presence in the nose does not contribute to the local inflammatory status.

To account for the bacterium's presence in the nose, the hypothesis of gastric-nasal transmission seems the most logical explanation since a significant number of patients with GERD and laryngopharyngeal reflux (LPR) also have *H. pylori* gastric colonization. It has been shown that the microorganism has a positive tropism for mucins[23] – and mucins cover and protect the sinus and mouth epithelia[24] – and is also able to invade epithelial cells.[25] The presence of *H. pylori* in the sinuses could then be regarded as a biomarker of the extent of LPR in the upper airway tract. However, at this time, we have no definitive proof of this, and we simply cannot rule out the possibility that the bacterium may use, in alternative or in conjunction, other routes to have its DNA reach the sinuses, for instance, via lymphatic or vascular transmission, from either the stomach or any other extra-digestive site.

Also, the fact that the presence of *H. pylori* in the sinuses apparently does not support a local pathogenic role does not entirely rule out the possibility that the bacterium may

influence the course of an inflammatory disease of the sinuses. *H. pylori* gastric infection is known to cause a vast array of systemic effects, including a strong immunologic response and gastrin and cytokine release from the stomach mucosa, all of which may indirectly affect chronic inflammation in any part of the respiratory system.[2] Definitive proof of the clinical relevance of these *H. pylori*–induced systemic effects is, however, still lacking.

CONCLUSION

Our results suggest that, regardless of how *H. pylori* reaches the sinuses, its presence there does not seem to contribute to the local inflammatory status of the respiratory mucosa. The fact that *H. pylori* could not be cultured from nose samples and is only present in its DNA form, suggests either a transient presence of parts of its genome in the sinuses, or, instead, what could be seen as a defensive adaptive reaction in preparation for a more or less lengthy stay at an inhospitable location, a change to a viable non-culturable form, from which *H. pylori* could hypothetically regain activity, to either play a role in the oral-oral route of transmission or in an eventual gastric re-infection.

Acknowledgements: We thank Hugo Dinis for assisting with the statistical analysis, Drª. Mónica Oleastro for confirming the PCR results, and Drª. Patrícia Fonseca for reviewing the manuscript.

Table 1. Status of gastric and nasal inflammation and *H. pylori* infection for all patients.

Patient	Breath test	Stomach				Sinus		
		Inflammation	*H. pylori* histology	*H. pylori* culture	*H. pylori* PCR - G / -S	Inflammation	*H. pylori* culture	*H. pylori* PCR - G / -S
1	Pos	Antrum: 2+	Antrum: 3+	Pos	Pos / Pos	2+	Neg	Pos / Pos
		Corpus: 2+	Corpus: 3+	Pos	Pos / Pos			
2	Neg	Antrum: NA	Antrum: NA	Neg	Neg / Neg	1+	Neg	Neg / Neg
		Corpus: NA	Corpus: NA	Neg	Neg / Pos			
3	Pos	Antrum: NA	Antrum: NA	NA	NA / NA	1+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 2+	Pos	Pos / Pos			
4	Pos	Antrum: 2+	Antrum: 0+	Pos	Pos / Pos	1+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 1+	Pos	Pos / Pos			
5	Neg	Antrum: 1+	Antrum: 0+	Neg	Neg / Pos	1+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 0+	Neg	Neg / Pos			
6	Pos	Antrum: 2+	Antrum: 3+	Pos	Pos / Pos	1+	Neg	Pos / Neg
		Corpus: 2+	Corpus: 2+	Pos	NA / Neg			
7	Pos	Antrum: NA	Antrum: NA	NA	NA/NA	2+	Neg	Neg / Neg
		Corpus: NA	Corpus: NA	NA	NA/NA			
8	Pos	Antrum: 2+	Antrum: 3+	Pos	Pos / Pos	2+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 2+	Pos	Pos / Pos			
9	Pos	Antrum: 1+	Antrum: 0+	Neg	Neg / Neg	1+	Neg	Neg / Neg
		Corpus: 1+	Corpus: 0+	Neg	Neg / Neg			
10	Neg	Antrum: 2+	Antrum: 1+	Pos	Pos / Neg	2+	Neg	Pos / Neg
		Corpus: 2+	Corpus: 0+	Pos	NA / Pos			
11	Pos	Antrum: 2+	Antrum: 2+	Pos	Neg / Pos	1+	Neg	Neg / Neg
		Corpus: 1+	Corpus: 0+	Pos	Neg / Neg			
12	Neg	Antrum: 1+	Antrum: 0+	Neg	Neg / Pos	2+	Neg	Pos / Pos
		Corpus: 1+	Corpus: 0+	Neg	Neg / Pos			
13	Pos	Antrum: 1+	Antrum: 0+	Pos	Pos / Pos	1+	Neg	NA / NA
		Corpus: NA	Corpus: NA	Pos	Pos / Neg			
14	Pos	Antrum: NA	Antrum: NA	NA	NA / NA	1+	Neg	Pos / Neg
		Corpus: NA	Corpus: NA	NA	NA / NA			

Neg=negative result. Pos=positive result. Inflammation grade: 0+= no inflammation; 1+= mild inflammation; 2+=moderate inflammation; 3+=severe inflammation. *H. pylori* histology: 0=no bacteria found; 1+=low density of bacteria; 2+= moderate density of bacteria; 3+=high density of bacteria. *H. pylori* PCR: G- genus pair of primers; S- species pair of primers. NA= data not available.

Table 2. Statistically significant correlations between the different variables tested.

Variables		Correlation Coefficient	p = value
PCR-G results in the nose	PCR-G results in the antrum	0.655	0.040
PCR-S results in the nose	Previous anti- *H. pylori* treatment	0.677	0.011
Breath test	Cultural identification of *H. pylori* in the stomach	0.625	0.030
Cultural identification of *H. pylori* in the stomach	Inflammatory status in the antrum	0.802	0.005
Histological identification of *H. pylori* in the antrum	Inflammatory status in the antrum	0.769	0.009
Histological identification of *H. pylori* in the antrum	Histological identification of *H. pylori* in the corpus	0.732	0.025
Breath test	PCR-G results in the corpus	0.655	0.040
Histological identification of *H. pylori* in the corpus	PCR-G results in the corpus	0.936	0.001
Cultural identification of *H. pylori* in the stomach	PCR-G results in the corpus	0.816	0.004
Cultural identification of *H. pylori* in the stomach	PCR-G results in the antrum	0.828	0.002
Histological identification of *H. pylori* in the corpus	PCR-G results in the antrum	0.763	0.017
PCR-G results in the antrum	PCR-G results in the corpus	1	-

CHAPTER FOUR

EPILOGUE

To comprehend why such a wide range of results and opposite conclusions concerning LPR, *H. pylori* and chronic inflammatory sinus disease exist in the medical literature, one must first understand the very nature of the digestive pathology at stake and the intrinsic value of each diagnostic test employed in its assessment. Reflux is an intermittent phenomenon, which may cause chronic upper respiratory tract inflammation through a direct and/or indirect effect, via vagal reflexes, on the respiratory mucosa. Pepsin seems to play a crucial role, and it has been shown that it remains active at pH values significantly higher than those in the stomach, which makes nonacid reflux probably no less pathogenic to the upper respiratory mucosa than acid reflux. Esophageal pH monitoring is still considered the gold standard for GERD diagnosis, but clinicians are still awaiting more appropriate tests for the specific diagnosis of LPR. If GERD is a hallmark of modern style living in developed countries, one can expect LPR to follow.

H. pylori, on the other hand, may or may not have the capacity to directly infect the respiratory mucosa. If its presence in the sinuses reflects gastric colonization, higher prevalence rates would be expected in the sinuses of subjects living in developing countries, and reports from those parts of the globe are indeed the most common. Besides geographic variance, the inconsistent data available also reflect the specificity and sensitivity of the different detection methods employed. Also, low bacterial numbers, unusual forms of the bacterium, and patchy and intermittent mucosal distribution are further confusing variables.

This thesis investigated the hypothesis that LPR and its contents, including *H. pylori*, are in some way associated with chronic inflammatory disease of the paranasal sinuses. In a case-control study of patients selected for sinus surgery, either owing to chronic

rhinosinusitis or concha bullosa, we assessed the specific role of individual LPR contents – *Helicobacter pylori* and pepsin – in the development of sinonasal inflammation. No evidence of a recent peptic reflux episode into the sinuses was found in any of the studied subjects, but *H. pylori* DNA was encountered evenly scattered through the sinuses, in both healthy (8% positive) and chronically inflamed mucosa (19% positive) samples, but this difference was not statistically significant.

In a cohort study of patients with chronic rhinosinusitis selected for sinus surgery, we investigated the role of *H. pylori* in the pathogenesis of sinonasal inflammation, and the bacterium's presence in the nose was compared to the gastric colonization pattern. We found that *H. pylori* DNA was present in the sinus mucosa of 15.4% of patients with chronic rhinosinusitis, all of them having concurrent *H. pylori* gastric infection. We also found that the germ's presence in the nose was unrelated to the degree of local inflammation, and the bacterium could not be cultured from any of the sinus tissue samples. This led us to conclude that *H. pylori* in the nose seems less likely a pathogen, but more of a tissue biomarker of reflux in the upper respiratory track.

Considering these results, the next research project will be to investigate an eventual presence of *H. pylori* DNA in the lachrymal sac of subjects with chronic obstructive disease of the lachrymal drainage system undergoing endoscopic endonasal dacryocystorhinostomy (DCR). Documentation of the presence of the germ's DNA in the lachrymal sac in a number of DCR patients would not only support a nasogenic origin for the obstructive disease of the lachrymal system but would also imply that reflux could even ascend to the nasolachrymal duct from the nose.

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REFERENCES CHAPTER ONE

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APPENDIX

Otolaryngology–Head and Neck Surgery (2006) 134, 67-72

ORIGINAL RESEARCH

Helicobacter Pylori and Laryngopharyngeal Reflux in Chronic Rhinosinusitis

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OBJECTIVE: Investigation of the potential role of several laryngopharyngeal reflux contents in sinus disease.

STUDY DESIGN AND SETTING: A controlled cohort analysis of *Helicobacter pylori*, pepsin and pepsinogen I in inflamed and non-inflamed sinonasal tissue. Fifteen patients, selected for surgery due to chronic medically refractory rhinosinusitis, had their pathologic sinus tissue analyzed for polymerase chain reaction detection of *H. pylori* DNA and assayed for pepsin and pepsinogen I tissue concentration levels. A control group of 5 patients undergoing surgery for anatomic sinonasal abnormalities provided non-inflammatory mucosa specimens for comparison.

RESULTS: *H. pylori* was found scattered in inflamed and non-inflamed mucosa, whereas sinonasal tissue pepsin/pepsinogen never rose above blood levels in both groups.

CONCLUSIONS: Evidence of intra-operative peptic reflux into the sinuses was not found. As *H. pylori* was similarly encountered in healthy and diseased sinus mucosa, it seemingly fails to support a pathogenic role for this organism in the sinuses.

EBM rating: B-2b

Gastric reflux has been incriminated recently in a myriad of laryngeal and other supra esophageal symptoms,[1,2] a much debated topic under the banner name of laryngopharyngeal reflux (LPR) disease. Patients with clinical LPR undergoing pH monitoring have documented acid reflux intermittently present in their pharynx, which can sometimes be traced as high in the airway tract as the nasopharynx,[3-5] with gastric pepsin being detected even in middle ear effusion of children with secretory otitis media.[6] Due to the protean nature of LPR symptoms, a host of controversies remain.[2] A hypothetical relation between LPR and chronic inflammation of the nose and sinuses was further added to the debate, in acknowledgment of the fact that both conditions intersect fairly consistently in clinical practice.[7-9] Reports of improvement of chronic sinusitis in children after anti-reflux therapy apparently support this relationship.[4,10,11] Omeprazole is able to reputably improve symptoms of refractory chronic sinusitis in adults.[12]

A number of pathophysiologic mechanisms for LPR symptoms have been proposed. Because *Helicobacter pylori* is prevalent in gastric contents, this organism has also been added to the list of potential role players in reflux-induced supra esophageal mucosa injury. *H. pylori* was declared a likely laryngeal pathogen[13] and its presence was also detected in diseased sinonasal tissue.[14,15] Thus far, however, no well-designed controlled studies have emerged to clarify the role of this organism in upper respiratory tract inflammation.

MATERIAL AND METHODS

Fifteen (11 male, 4 female) patients, aged 18 to 79 (mean = 49.9 years), requiring endoscopic sinus surgery due to medically recalcitrant chronic rhinosinusitis involving the anterior and the posterior ethmoid on at least one nasal cavity, with or without simultaneous sphenoid sinus disease, were enrolled as the Study Group (Fig 1). Informed consent was obtained from each patient and the study protocol was subject to approval by the institutional ethics committee. All patients had their biochemical and hematological profiles within the normal range, with the exception of mild to moderate eosinophilia found in some atopic patients. Patients with cystic fibrosis, immotile cilia syndrome, and known immunodeficiencies were excluded. In all patients the inflammatory nature of the disease was confirmed subsequently by histopathologic examination.

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0194-5998/$32.00
doi:10.1016/j.otohns.2005.10.013

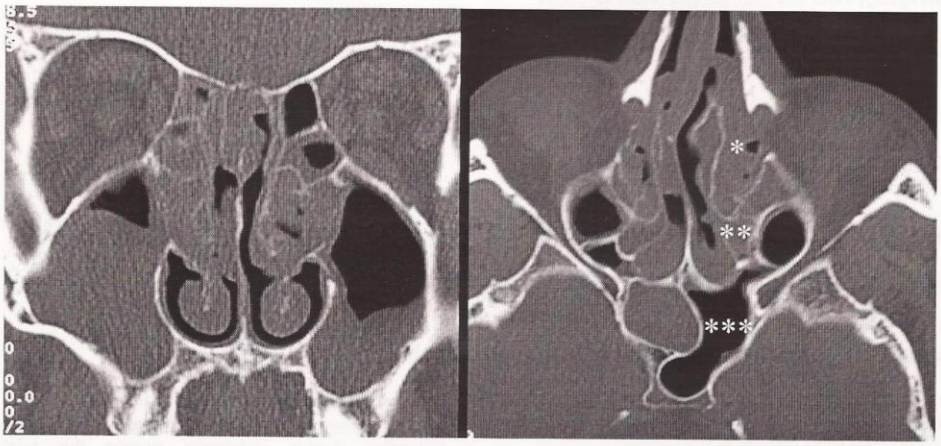

Figure 1 CT scan of a typical Study Group patient, with extensive chronic inflammatory sinus disease requiring surgery for bilateral anterior (*left-hand side on the axial plane) and posterior ethmoid (**) sinuses pathology, with sphenoid sinusitis on the right-hand side. A total right sphenoethmoidectomy with total left ethmoidectomy was carried out, which provided diseased tissue samples from the right and left anterior ethmoids, right and left posterior ethmoids, as well as right sphenoid sinus. The left sphenoid sinus (***), spared surgically, failed to contribute a site sample to the investigation.

Five (1 male, 4 female) patients, aged 22 to 72 (mean = 37.6 years), requiring endoscopic sinus surgery due to endonasal anatomic variations (symptomatic concha bullosa) that did not produce CT scan-detectable sinus inflammation, were enrolled as the Control Group (Fig 2).

In the immediate pre-operative period of all patients, drug therapy for any underlying condition was allowed, including systemic steroids for asthma management. Excluded from the investigation, however, were patients who undertook antimicrobial therapy of any kind, including in prophylaxis, in the last weeks before surgery. Also, no patient had undergone treatment with antacids, histamine$_2$-receptor antagonists, prokinetic agents, or proton-pump inhibitors in the recent pre-operative period. No

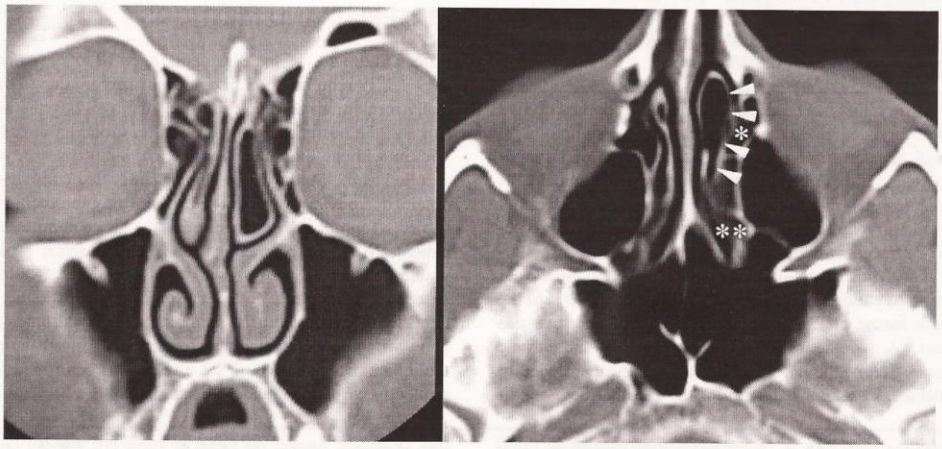

Figure 2 CT scan of a typical Control Group patient, requiring surgery for a symptomatic left concha bullosa (*axial plane), but without imaging evidence of inflammation in any part of the sinuses. The meatal mucosa of the partially resected middle turbinate, signaled by the arrows, was labeled non-inflamed anterior ethmoid sample, whereas a posterior ethmoid non-inflamed mucosa sample from the same side (**) was collected through a small opening at the basal lamella. The right side of the nose was not surgically manipulated, thus failing to contribute any site sample to the investigation.

patient had a history of prior fundoplication or Barrett's esophagus.

Surgery was carried out under general anesthesia with orotracheal intubation. No patient required nasogastric tube placement and vomiting did not occur, pre- or intra-operatively, according to anesthesia chart review.

Blood samples (7 ml) were collected immediately before induction of general anesthesia from both groups. Serum was separated by centrifugation and immediately frozen at –70°C until assayed. Concentrations of pepsin and pepsinogen I were determined by an immunoassay with rooster polyclonal antibodies to purified human pepsin,[16] or radioimmunoassay,[17] respectively.

Preceding the intervention, routine vasoconstriction of the nasal mucosa to minimize intra-operative bleeding was carried out, including infiltration at critical sites using 1% Xylocaine with 1:100,000 epinephrine.

During surgery, tissue samples of sinonasal mucosa were collected in the study group at the anterior ethmoid (including material taken from the uncinate process, ethmoidal bulla and meatal portion of the middle turbinate), the posterior ethmoid, and eventually, the sphenoid sinus. The extent of the pathology on the CT scans determined which sites were to be surgically addressed, but pathology at anterior and posterior ethmoid on at least one side of the nose, with or without associated sphenoid disease, was required for study enrollment (Fig 1).

In the control group, tissue samples were collected from the meatal portion of the middle turbinate of the concha bullosa, which served as anterior ethmoid specimen, and from a donor site of healthy, posterior ethmoid, mucosa on the same side of the nose (Fig 2). The latter specimens were collected through a small opening at the basal lamella, with the utmost care to minimize collateral damage to nasal anatomy and function. One or both sides of the nose were addressed this way according to pre-determined require-to-treat surgical planning. Site inflammation was ruled out subsequently by histopathologic examination, whereas post-operative endoscopic control warranted that proper healing occurred in all operated sides.

In both groups, the mucosa specimens collected at all the different sinonasal sites were immediately frozen at –70°C and assayed subsequently for pepsin and pepsinogen I concentration levels, using an immunoassay with rooster polyclonal antibodies to purified human pepsin[16] and radioimmunoassay methodology,[17] and for polymerase chain reaction (PCR) extraction and amplification of genomic *H. pylori* DNA.[18] The method, described elsewhere,[17] employed the PCR-based microtiter plate hybridization technique with primers targeted to an *ureA* gene segment of *H. pylori*. The sensitivity of the method is 10 bacterial cells, with 100% specificity.

Statistical analysis employed the following tests according to specific requirements: the Mann-Whitney test, Wilcoxon signed-rank test, paired-samples *t*-test, Pearson's χ^2 test, Fisher's test, and the McNemar test. For all, a significance level was set at $P = 0.05$.

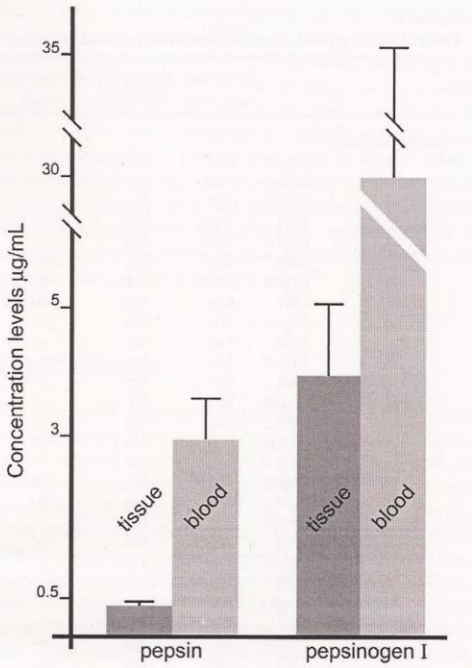

Figure 3 Mean concentration levels and SD of pepsin and pepsinogen I in sinonasal tissue and serum in the Study Group.

RESULTS

Control Group patients are mostly female and younger in age and Study Group patients seem biased toward the male gender and have higher rates of allergy, asthma, and aspirin intolerance comorbidity. However, statistic analysis comparisons of the demographics of both groups are affected by the small number of patients in the Control Group.

Figure 3 illustrates the Study Group mean concentration results and SD of pepsin and pepsinogen I in serum and in sinonasal tissue. The tissue:blood ratio for pepsin and pepsinogen I was 0.17. No statistically significant differences were found between patients and controls regarding blood and mucosa pepsin and pepsinogen I values. Gender, site, nasal side, disease extension, and co-presence of allergy, asthma, and aspirin intolerance, were not found to have an influence on the sinus tissue concentration levels of both enzymes. It was found, however, that age was an influence, as older patients tend to have higher sinonasal concentrations of pepsin ($P = 0.007$) and pepsinogen I ($P = 0.03$).

Table 1
Helicobacter pylori distribution in sinonasal mucosa

		Anterior ethmoid	Posterior ethmoid	Sphenoid sinus
Study patients				
1	Right	No	Yes	–
	Left	Yes	No	–
2	Right	No	No	–
	Left	Yes	Yes	–
3	Right	No	No	–
	Left	No	No	No
4	Right	Yes	Yes	No
	Left	No	Yes	No
5	Right	No	No	–
	Left	No	No	–
6	Right	No	No	–
	Left	No	No	–
7	Right	No	No	–
	Left	No	No	–
8	Right	No	No	No
	Left	No	No	–
9	Right	No	No	No
	Left	No	No	No
10	Right	No	No	–
	Left	No	No	–
11	Right	No	Yes	–
	Left	No	Yes	–
12	Right	No	Yes	–
	Left	No	Yes	–
13	Right	No	No	–
	Left	No	No	No
14	Right	No	No	–
	Left	No	No	–
15	Right	No	Yes	No
	Left	No	Yes	No
Control patients				
1	Right	No	No	–
	Left	–	–	–
2	Right	–	–	–
	Left	No	Yes	–
3	Right	No	No	–
	Left	No	No	–
4	Right	No	No	–
	Left	–	–	–
5	Right	No	No	–
	Left	–	–	–

Yes, positive for H. pylori DNA at this site; *No*, negative for H. pylori DNA at this site; –, no surgery required at this site.

Table 1 shows the results of *H. pylori* distribution per patient and surgical site in all patients. From the 69 tissue samples collected in the Study Group, 19% were positive for *H. pylori* infection, and of the 12 tissue samples collected in the Control Group, 8% were *H. pylori*-positive. No statistically significant differences were encountered in *H. pylori* colonization between diseased mucosa and control sinonasal tissue. In both groups, no variable, such as age, gender, nasal side, site, disease extension, co-presence of allergy, asthma, or aspirin intolerance, was found to relate to bacterial colonization. Even concomitant pepsin and pepsinogen I tissue levels were not found to statistically relate to *H. pylori* presence in any specific site in both groups.

DISCUSSION

Our study shows that the concentrations of pepsin and pepsinogen I in the sinuses are no higher than the pepsin and pepsinogen serum levels, with no single mucosal result ever exceeding blood concentrations. Because the extracellular fluid in chronically inflamed sinonasal mucosa results from plasma transudation processes, we needed to clarify if the pepsin levels detected at the mucosa were the result of pepsin from gastric juice or pepsin derived from plasma pepsinogen. The latter seems to be the case, as pepsin tissue levels are compatible with plasma transudation of pepsinogen into the sinus mucosa rather than a gastric reflux transport of the enzyme into the nose, in which case values up to 1,000-fold the serum levels can be expected.[6] This finding does not preclude the possibility that gastric contents may reflux into the nasal cavity in LPR patients. It merely states that no evidence of refluxate reaching the nose and sinuses could be documented during the time these patients were in surgery under general anesthesia. This conclusion is not surprising because LPR patients are typically upright-position daytime refluxers,[2] and as few as 3 reflux episodes/week are apparently sufficient to produce ongoing inflammation at the highly sensitive supra esophageal mucosa.[1,2]

Our study also shows that *H. pylori* is present in the sinonasal mucosa of a significant number of chronic rhinosinusitis surgical specimens. These results are in agreement with data published previously,[14,15] the occurrence perhaps being even more prevalent than reported.[14] The fact that, in our series, pre-operative antimicrobial prophylaxis was avoided and no patient was taking H_2-receptor antagonists or proton-pump inhibitors before surgery, may have enhanced the diagnosis sensitivity. The study design also allowed for a mapping distribution of the organism within the sinonasal cavity, which did not show any particular pattern of colonization. Therefore, the posterior sinuses (i.e., posterior ethmoid and sphenoid sinus) are at no greater risk of being colonized by the organism than the anterior ethmoid, as gastric backflow mechanism would have it. Unless unforeseen factors have biased our data (i.e., lidocaine has been shown to inhibit the in vitro growth of the organism), nasal colonization by *H. pylori* in chronic rhinosinusitis has a patchy distribution and does not seem to follow an established pattern.

The pathophysiology of LPR disease is a much debated topic, with mechanisms direct peptic-acid injury and triggered neurophysiologic changes, cited most frequently.[2,9] The possibility that *H. pylori* may also play an etiopathogenic role was only put forth very recently.[14,15] This organism is prevalent in the gastric contents of a large number of humans worldwide.[19] Once infected, adults will maintain lifelong *H. pylori* colonization, until eradication by specific

therapy.[19] Gastric infection is characterized by a non-invasive mucosal surface attachment of the bacterium that triggers a specific host response, involving neutrophils, T and B lymphocytes, plasma cells, and macrophages, ultimately leading to tissue damage.[19] Although expressed clinically in highly variable ways, virtually all *H. pylori*-infected persons have chronic superficial gastritis, often displaying a patchy distribution at the stomach mucosal surface[19] (not unlike the colonization pattern found in the sinuses).

Because a significant number of gastroesophageal reflux disease (GERD) patients are infected with *H. pylori*, and the infection seems to disturb lower oesophageal sphincter function,[19] the hypothesis of a refluxate-conveyed bacterial seeding of the supra esophageal mucosa in LPR patients is perhaps not too far-fetched. It has been suggested that the reflux of infected gastric juice into the nasopharynx may produce airway mucosal edema and inflammation from the combined activity of peptic-acid injury *and* local *H. pylori* infection.[14] A direct relation between the severity of the mucosal inflammation and sinus *H. pylori* colonization rates can even be argued for, because sinus surgical failure patients, with surgically as well as medically recalcitrant pathology, as opposed to medical-only failures from our studied population, thus apparently with more severe sinus disease, have been shown to suffer from an increased prevalence of LPR.[5] The <100%-positive bacterial identification rate scenario we have identified in the inflamed sinus mucosa could also be mimicking what is otherwise reported to occur at other colonization sites in the body: that repeated and extensive sampling is required frequently to ensure that a certain diagnostic methodology does not miss out on the *H. pylori* mucosal infection. If the T helper (Th) 1-associated *H. pylori*-inflammation at the stomach[19] seems at odds with the Th2 eosinophic pattern that histopathologically hallmarks chronic rhinosinusitis at the nose, one should acknowledge possible similarities between the latter and an entity called eosinophilic esophagitis.[20] The latter often mimics GERD but is characterized by a lack of response to acid suppression,[20] a feature shared with severe LPR.

Any relation, however, remains speculative. At no other location than the gastric mucosa was *H. pylori* found to cause disease, although the organism was also detected in saliva, dental plaque, adenoids, and tonsils.[19] The presence of the bacterium in the sinuses may even be the result of a direct transmission of the infection from any of these sites into the nasal cavity. If the role of *H. pylori* in extragastric locations is presently unclear, some believe they serve as reservoir sites for gastric re-infection, as recurrence after antimicrobial therapy is not uncommon.[19] The fact that in our study, *H. pylori* colonization was not statistically found to be more common in sinusitis patients than in controls, lends credibility to the *nose as a reservoir* thesis. Whether the nose acts as a permanent or a transient extra-gastric settling place for the organism is not known, the latter hypothesis agreeing more with the pattern of inconstant identification of the organism in sinonasal mucosa we have found. Thus, potential clinical benefits of anti-reflux therapy in chronic rhinosinusitis[4,10-12] seems more likely to result from the suppression of the LPR-induced, peptic-acid or neurologic, pathogenic effects on the upper airway mucosa, rather than from an influence on the nose and sinuses' *H. pylori* colonization. Although we acknowledge that our studied population is perhaps too small in size to allow a definitive word on this matter, our conclusions are nevertheless remarkably consistent with the recent concept that, when in co-morbidity, the pathogenic mechanisms of *H. pylori* infection and reflux disease probably run in a parallel fashion, independent from each other.[19]

CONCLUSION

If the role of *H. pylori* infection in gastroduodenal disease is unquestionable, the manner by which the organism is transmitted remains unclear. To the established fecal–oral and oral–oral routes, newer routes of transmission have been proposed such as gastric–oral and, more recently, gastric–nasal transmission, the latter the aim of our investigation. *H. pylori* DNA may be encountered in chronically inflamed sinonasal mucosa, possibly as a manifestation of LPR disease, although at this time we can only speculate on the role of the bacterium at this particular location. The possibility that it plays some etiopathogenic role in rhinosinusitis can not be dismissed too lightly, as theoretically there is some valid reasoning behind it. Our data, however, does not seem to substantiate a pathogenic presence of the organism in the nose. At the sinonasal mucosa *H. pylori* more likely has a reservoir function similar to other extragastric, upper aerodigestive, site infections, possibly contributing to the oral–oral route of transmission or instigating gastric reinfection.

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ORIGINAL CONTRIBUTION

OPEN ACCESS

Helicobacter pylori in both the sinuses and the stomach*

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Rhinology Online, Vol 1: 194 - 200, 2018
http://doi.org/10.4193/RHINOL/18.035

***Received for publication:**
July 24, 2018
Accepted: October 26, 2018

Abstract

Background: The role played by *Helicobacter pylori* in the sinuses, and its association with the same organism's gastric infection, are still unclear.

Methods: In order to compare H.pylori colonization patterns in the nose and stomach we conducted a cohort analysis of 14 patients, eligible for sinus surgery due to chronic medically refractory rhinosinusitis, who were tested for simultaneous presence of *H. pylori*, by histology, culture and polymerase chain reaction, in pathologic sinus tissue collected during surgery and in gastric mucosa obtained through gastroduodenal endoscopy.

Results: *H. pylori* DNA was found in the sinus mucosa of 15.4% of patients with chronic rhinosinusitis, and all of them showed concurrent *H. pylori* stomach infection. Sinus colonization was not found without simultaneous gastric colonization, although most patients with gastric infection did not have the bacterial DNA in their sinuses. *H. pylori*'s presence in the nose was not associated with local inflammatory status, and no cultures could be obtained from any of the sinus tissue samples, including those positive for *H. pylori* DNA.

Conclusions: Only *H. pylori* DNA, and not the culturable active form of the microorganism, could be found in the sinus mucosa of some patients with *H. pylori* gastric infection. We could not find evidence, however, that the bacterium's presence in the nose contributes to local mucosal inflammation.

Key Words: chronic rhinosinusitis, *Helicobacter pylori,* gastric infection

Introduction

Helicobacter pylori DNA has been detected in a number of extra-gastric locations, such as the oral cavity, tonsils and adenoids[1,2], and even the middle ear and paranasal sinuses[2-15]. However, the significance of the microorganism's presence at these aero- digestive and respiratory sites is still unclear. It is believed that the oral cavity is an important reservoir for *H. pylori*, that contributes to the oral-oral route of transmission and acts as a source of stomach re-infection[1]. Others suggest that the bacterium may be capable of causing damage to the aero-digestive and respiratory mucosa in the same way it does to the gastric mucosa[1]. However, this capacity for extra-gastric disease remains unproved, and some authors argue that it even seems unlikely.

In order to initiate tissue damage, not only does *H. pylori* require a set of events unique to the gastric milieu, but also culturable active forms of *H. pylori* have only been recovered outside the stomach in the aero-digestive tract in the vicinity of the upper esophagus (i.e. in tracheal secretions in intubated patients), and never as far away as the bronchi, lung, or the upper respiratory tract[2]. That far from the stomach, only the microorganism's DNA suggests the presence of *H. pylori* at such locations.

We conducted the present investigation in order to see how *H. pylori* colonization patterns in the nose and stomach are associated in chronic rhinosinusitis patients, as a way to also provide information on the eventual existence of an interaction between two seemingly unconnected pathologies, one in the digestive

tract and the other in the upper respiratory tract. At the same time, we assessed if this known gastric pathogen has the capacity to also inflict direct injury to the sinus mucosa.

Materials and Methods

Patients

A total of 14 adult patients, 8 male and 6 female, aged 30 to 68 years (mean 48.1 years), requiring endoscopic sinus surgery due to medically recalcitrant chronic rhinosinusitis, with and without nasal polyposis, were enrolled in a consecutive, unselected manner. Informed consent was obtained from each patient, and the study protocol was approved by the institutional ethics committee. All patients had their biochemical and hematological profiles within the normal range, with the exception of mild-to-moderate eosinophilia in some atopic patients. Patients with cystic fibrosis, immotile cilia syndrome, and known immunodeficiencies were excluded. The inflammatory nature of the disease was subsequently confirmed by histopathologic examination in all patients enrolled.

Drug therapy for any underlying condition was allowed, including systemic steroids for asthma management. Antibiotic use in the 3 months prior to surgery was also noted. Moreover, patients were specifically asked about their history of prior *H. pylori* infection diagnosis and treatment, as well as current medication with antacids, histamine 2 -receptor antagonists, prokinetic agents, or proton-pump inhibitors (PPI's).

Procedures

In the immediate pre-operative period (from 1 to 10 days pre-op), all subjects underwent a 13C-labelled urea breath test. Surgery was performed under general anesthesia, with orotracheal intubation. Routine vasoconstriction of the nasal mucosa was carried out to minimize intra-operative bleeding, including infiltration, at critical sites, with 1% xylocaine with 1:100,000 epinephrine.

The extent of the pathology on the computed tomography scans determined which sites were to be surgically approached. All patients required bilateral procedures due to roughly symmetrical extension of the pathology, according to the Lund-Mc-Kay classification[16]. During surgery, tissue samples of diseased sinonasal mucosa were collected at the anterior ethmoid (including the uncinate process, ethmoidal bulla, and meatal portion of the middle turbinate), and the posterior ethmoid sinus from one, randomly chosen, nasal cavity. The collected sinus specimens were subjected to: 1) histopathological examination, to assess the overall degree of local inflammation (graded as 0+, no inflammation; 1+, mild; 2+, moderate; and 3+, severe inflammation, depending on the inflammatory infiltrate cell density[17]); 2) microbiological culture, to isolate *H. pylori*; and 3) polymerase chain reaction (PCR) to amplify genomic *H. pylori* DNA.

Enrolment required that the patients also agree to undergo a simultaneous upper gastrointestinal endoscopy, irrespectively of digestive symptoms. Biopsies were taken from the stomach mucosa lining, either from endoscopic-assessed suspected sites or iteratively, from the lesser or greater curvature of the antrum and the greater curvature of the corpus. Patients underwent gastroduodenal endoscopy either simultaneously with the sinonasal mucosa sampling (intra-operatively, under general anesthesia, while waiting for the vasoconstriction effect to occur in the nose) or up to two weeks post sinus surgery. The collected gastric biopsy specimens from the two sites, the antrum and the corpus, were analyzed by: 1) histopathology, to assess the overall degree of local inflammation (graded as 0+, absent; 1+, mild; 2+, moderate; 3+, severe inflammation, depending on the inflammatory infiltrate cell density); 2) histology to identify *H. pylori* in Warthin-Starry silver stained gastric mucosal biopsies, (also graded as 0+, no bacteria found; 1+, mild ; 2+, moderate; 3+, severe density colonization); and 3) microbiological culture of gastric biopsy specimens specific for *H. pylori*.

All sinus and stomach tissue samples were collected aseptically, transported to the laboratory at 4ºC in Portagerm pylori (bioMérieux, France), and processed less than four hours after collection. For microbiological culture, a drop of tissue macerate was plated onto *H. pylori*-selective medium (Brucella supplemented with 10% horse blood and Brucella supplemented with 10% horse blood and Oxoid *H. pylori*-selective supplement (Dent)) and incubated at 37ºC in a microaerophilic atmosphere (Campygen, Oxoid) for up to 15 days. Colonies were then tested for urease, catalase and oxidase, and motility and were stained with Gram stain.

Polymerase chain reaction

For PCR testing, two sets of primers targeting the 16S rRNA and 23S rRNA genes of *H. pylori* were used, chosen to maximize detection as together they target sequences common to virtually all *H. pylori* strains[18,19]. The first primer pair, PCR-G, amplifies a 780bp fragment from the 16S rRNA gene specific for the *Helicobacter* genus, Helico F – 5′ CTATGACGGGTATCCGGC 3′ and Helico R – 5′ CTCACGACACGAGCTGAC 3′[18]. The second primer pair, PCR-S, amplifies a 267 bp fragment from the 23S rRNA gene of the *H. pylori*, HPYS - 5 ′ CGCATGATATTCCCATTAGCAGT 3′ and HPYA - 5′ AGGTTAAGAGGATGCGTCAGTC 3′[19]. Ethidium bromide-stained agarose gel electrophoresis was employed for separating the PCR products according to their size. When interpreting the results, we considered a positive genus-specific PCR test a sign of presence of DNA for one of the various Helicobacter species, whilst the combination of a positive genus-specific with a positive species-specific PCR test was taken as a clear sign of presence of the actual *H. pylori* species.

Table 1. Status of gastric and nasal inflammation and *H. pylori* infection for all patients.

Patient	Breath test	Stomach				Sinus		
		Inflammation	*H. pylori* histology	*H. pylori* culture	*H. pylori* PCR - G / -S	Inflammation	*H. pylori* culture	*H. pylori* PCR - G / -S
1	Pos	Antrum: 2+	Antrum: 3+	Pos	Pos / Pos	2+	Neg	Pos / Pos
		Corpus: 2+	Corpus: 3+	Pos	Pos / Pos			
2	Neg	Antrum: NA	Antrum: NA	Neg	Neg / Neg	1+	Neg	Neg / Neg
		Corpus: NA	Corpus: NA	Neg	Neg / Pos			
3	Pos	Antrum: NA	Antrum: NA	NA	NA / NA	1+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 2+	Pos	Pos / Pos			
4	Pos	Antrum: 2+	Antrum: 0+	Pos	Pos / Pos	1+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 1+	Pos	Pos / Pos			
5	Neg	Antrum: 1+	Antrum: 0+	Neg	Neg / Pos	1+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 0+	Neg	Neg / Pos			
6	Pos	Antrum: 2+	Antrum: 3+	Pos	Pos / Pos	1+	Neg	Pos / Neg
		Corpus: 2+	Corpus: 2+	Pos	NA / Neg			
7	Pos	Antrum: NA	Antrum: NA	NA	NA/NA	2+	Neg	Neg / Neg
		Corpus: NA	Corpus: NA	NA	NA/NA			
8	Pos	Antrum: 2+	Antrum: 3+	Pos	Pos / Pos	2+	Neg	Pos / Neg
		Corpus: 1+	Corpus: 2+	Pos	Pos / Pos			
9	Pos	Antrum: 1+	Antrum: 0+	Neg	Neg / Neg	1+	Neg	Neg / Neg
		Corpus: 1+	Corpus: 0+	Neg	Neg / Neg			
10	Neg	Antrum: 2+	Antrum: 1+	Pos	Pos / Neg	2+	Neg	Pos / Neg
		Corpus: 2+	Corpus: 0+	Pos	NA / Pos			
11	Pos	Antrum: 2+	Antrum: 2+	Pos	Neg / Pos	1+	Neg	Neg / Neg
		Corpus: 1+	Corpus: 0+	Pos	Neg / Neg			
12	Neg	Antrum: 1+	Antrum: 0+	Neg	Neg / Pos	2+	Neg	Pos / Pos
		Corpus: 1+	Corpus: 0+	Neg	Neg / Pos			
13	Pos	Antrum: 1+	Antrum: 0+	Pos	Pos / Pos	1+	Neg	NA / NA
		Corpus: NA	Corpus: NA	Pos	Pos / Neg			
14	Pos	Antrum: NA	Antrum: NA	NA	NA / NA	1+	Neg	Pos / Neg
		Corpus: NA	Corpus: NA	NA	NA / NA			

Neg=negative result. Pos=positive result. Inflammation grade: 0+= no inflammation; 1+= mild inflammation; 2+=moderate inflammation; 3+=severe inflammation. *H. pylori* histology: 0=no bacteria found; 1+=low density of bacteria; 2+= moderate density of bacteria; 3+=high density of bacteria. *H. pylori* PCR: G- genus pair of primers; S- species pair of primers. NA= data not available.

Histology

For histological identification of *H. pylori* in paraffin sections of sinus tissue, the modified Warthin-Starry silver staining kit (Merck, Germany) was used.

The grading of the histological material, as well as all the molecular biology procedures, were performed after the identification of the samples was masked.

Statistics

Statistical analysis employed descriptive statistics and the Spearman's rank correlation coefficient (Spearman's rho).

Results

The study group included 8 patients with asthma, 6 with allergic rhinitis, and 2 with intolerance to nonsteroidal anti-inflammatory drugs (NSAIDs). Other co-morbidities included: hypertension (n=2), hypothyroidism (n=1), goiter (n=1), angioma of the liver (n=1), ankylosing spondylitis (n=1), fibromyalgia while on NSAID's (n=1), gastroesophageal reflux disease (GERD) (n=2) and chronic gastritis (n=1). Two patients were undergoing revision sinus surgery, and three admitted having taken an oral antibiotic in the immediate three months prior to surgery. One subject had history of *H. pylori* gastric infection treatment, while five

Table 2. Statistically significant correlations between the different variables tested.

Variables		Correlation Coefficient	p = value
PCR-G results in the nose	PCR-G results in the antrum	0.655	0.040
PCR-S results in the nose	Previous anti- *H. pylori* treatment	0.677	0.011
Breath test	Cultural identification of *H. pylori* in the stomach	0.625	0.030
Cultural identification of *H. pylori* in the stomach	Inflammatory status in the antrum	0.802	0.005
Histological identification of *H. pylori* in the antrum	Inflammatory status in the antrum	0.769	0.009
Histological identification of *H. pylori* in the antrum	Histological identification of *H. pylori* in the corpus	0.732	0.025
Breath test	PCR-G results in the corpus	0.655	0.040
Histological identification of *H. pylori* in the corpus	PCR-G results in the corpus	0.936	0.001
Cultural identification of *H. pylori* in the stomach	PCR-G results in the corpus	0.816	0.004
Cultural identification of *H. pylori* in the stomach	PCR-G results in the antrum	0.828	0.002
Histological identification of *H. pylori* in the corpus	PCR-G results in the antrum	0.763	0.017
PCR-G results in the antrum	PCR-G results in the corpus	1	-

were taking a PPI until the day before surgery. The patient with ankylosing spondylitis was under treatment with sulfasalazine at the time of the surgical procedure, and the stiffness of his entire spine was so severe that the gastroenterologist was unable to perform the required gastroduodenal endoscopy, even under general anesthesia. Another patient underwent upper gastrointestinal endoscopy outside of the time frame imposed by the strict study criteria (either intra-op or up to two weeks post sinus surgery), so the gastric results were excluded from the analysis. Two patients had histology results for one gastric site only, and in one patient the gastric histological results were altogether absent.

The inflammatory status of the sinus mucosa at the time of surgery for each patient is displayed in Table 1. Severe inflammation was not encountered in any subject; moderate inflammation was found in 50% (n=5) of the asthmatics and in 16.6 % (n=1) of the non-asthmatics, whilst mild inflammation was encountered in 50% (n=4) of the asthmatics and in 83.3 % (n=5) of the non-asthmatics. A total of 60% (n=3) of patients in the moderate sinus inflammation group had allergic rhinitis, while mild inflammation was observed in 50% (n=3) of the patients with a diagnosis of allergic rhinitis. All patients with intolerance to NSAIDs in the study group displayed moderate sinus inflammation.

Moderate sinus inflammation was also encountered in 66.7% (n=2) of the patients who had taken an antimicrobial agent in the three months prior to surgery, whilst mild inflammation was mostly found (72.7%, n=8) in patients who had not recently needed antibiotic treatment.

The only patient who had prior treatment for *H. pylori* gastric infection displayed moderate sinus inflammation, while, among the five patients who were on PPI's at the time of surgery, three showed mild sinus inflammation and two moderate sinus inflammation.

Table 1 shows the results of the gastric and nasal inflammatory and *H. pylori* infection status for all patients.

All patients were found to have some degree of gastric inflammation, fitting the histopathological diagnosis of chronic non-atrophic gastritis (active or non-active), even when the endoscopist failed to identify inflammation and performed iterative biopsies. A total of 60% of the antrum biopsies showed moderate inflammation, with mild inflammation occurring in 40%, whereas in corpus biopsies 70% had mild and 30% moderate inflammation.

Histological identification of *H. pylori* in gastric mucosa biopsies of the antrum was negative in 50% of the cases (n=5), revealing mild infection in 10% (n=1), moderate infection in 10% (n=1), and severe infection in 30% of the cases (n=3). For the corpus biopsies, the results were negative in 50% (n=5), with mild infection in 10% (n=1), moderate in 30% (n=3), and severe infection in 10% (n=1).

The results of cultures of gastric mucosa biopsies were positive for *H. pylori* in 66.7% of the cases (n=8), whilst the PCR-G test was positive in 54.5% (n=6) of the antrum samples and in 50% (n=5) of the corpus samples, and the PCR-S test was positive in 72.7% (n=8) of the antrum samples and in 66.7% (n=8) of the corpus samples.

A total of 66.6% of the patients receiving PPI treatment were *H. pylori* negative.

Table 2 shows the correlations between the variables found to

have statistical significance.

Regarding *H. pylori* presence in the sinonasal mucosa, the species-specific PCR identification was positive in only two cases (15.4%), although 69.2% had sinonasal samples with positive genus-specific PCR results. The first case of positive *H. pylori* DNA in the sinuses had a positive breath urea test and tested positive for *H. pylori* in the stomach by histology, bacterial culture and PCR. The second case, however, had a negative breath test and tested negative for gastric *H. pylori* by histology and bacterial culture, but the species-specific PCR identification test in the stomach was positive (result confirmed at a different laboratory). All attempts to grow *H. pylori* in culture medium from sinonasal samples failed.

Discussion

The characteristics of our investigation, with patients undergoing simultaneous extensive sinus and stomach tissue sampling, and with various tests concurrently performed, necessarily restricts the number of patients enrolled in the study, and as a result may not be able to show statistical significance. The available data, however, are sufficient to allow for important conclusions.

Since urease production is a hallmark of active gastric infection, it is perhaps no surprise that most, but not all, patients in our study with a positive urease breath test had indeed *H. pylori* gastric infection, confirmed by histopathology, culture, and/or PCR testing. The breath test was positive in one case where *H. pylori* was detected in the sinuses, but was negative in the other. As the majority of patients in our study had positive breath tests and no evidence of *H. pylori* in their sinuses, this apparently renders the test unsuitable for the specific identification of the bacterium in the nose.

Our data actually reveals that, in spite of the relevant number of patients with positive Helicobacter-genus DNA detected by PCR in the sinus mucosa (which certainly merits separate investigation), specifically *H. pylori* DNA was only found in the sinonasal mucosa of about 15.4% of chronic rhinosinusitis surgical patients. These results are in agreement with previously published data[3-15]. Admittedly, this prevalence could indeed be greater if the bacterium in the nose would follow a similar mosaic pattern of mucosal infection as in the stomach, with patches of diseased mucosa alternating with non-infected normal mucosa, in which case it would require repeated and extensive sampling to allow for a positive *H. pylori* result (five different sites, according to some)[20].

Both cases with *H. pylori* DNA in the sinuses had *H. pylori* DNA simultaneously present in their stomachs, but only about 25-28.6% of the patients with positive gastric species-specific PCR tests had *H. pylori* DNA in their sinuses. This suggests that if the bacterium is to be encountered in the nose, its DNA has to also be present in the stomach, but that not all *H. pylori* gastric infections are necessarily associated with *H. pylori* colonization of the sinuses.

Our data show that while there is a positive correlation between cultural and histological identification of *H. pylori* in the stomach and gastric inflammation, we found no such correlation between *H. pylori* and site inflammation in the nose (Table 2). Therefore, it is perhaps not farfetched to admit that *H. pylori* presence in the nose and sinuses does not contribute to local mucosal inflammation.

The diagnosis of allergic rhinitis, asthma or intolerance to NSAIDs, was not found to statistically relate to either positive or negative sinonasal *H. pylori* results, suggesting that sinonasal *H. pylori* colonization may occur regardless these co-morbidities are present or not.

Critically, *H. pylori* could only be recovered from the nose in the DNA form, as all the attempts to culture the bacterium from nasosinusal sites failed. This inability to culture *H. pylori* could be due to the presence of too few microorganisms to be detected, or the simultaneous presence of too many types of other bacteria in the nose that inhibit growth of *H. pylori*. However, it has been shown that the bacterium can be cultured from adverse environments such as the air sampled during vomiting or from a tracheostomy tube[2]. So it is admissible that the reason may not have to do with the method but with the possibility that, in the sinuses, either the organism is represented just by fragments of its DNA and that these are destined to transiently remain there just for a limited time, or the microorganism is, in fact, in a dormant state that precludes culture, and is destined to remain for a long time in the sinuses.

The bacterium is, indeed, known to be able to resist harsh environments by changing to a dormant, inactive state, a non-culturable coccoid form that could still be potentially viable, later on, in the stomach[21,22]. It is therefore possible that *H. pylori* may lay dormant for long periods of time, using the nose and the sinuses as reservoirs, waiting for an eventual return to an active form, either to cause gastric re-infection or to participate in the oral-oral route of transmission. The fact that in a previous study no statistical difference was observed between *H. pylori* nasal colonization in patients with sinusitis when compared to the control group[6], lends credibility to the 'nose as a reservoir' thesis, and reinforces our conviction that the bacterium's presence in the nose does not contribute to the local inflammatory status. To account for the bacterium's presence in the nose, the hypothesis of gastric-nasal transmission seems the most logical explanation since a significant number of patients with GERD and laryngopharyngeal reflux (LPR) also have *H. pylori* gastric colonization. It has been shown that the microorganism has a positive tropism for mucins[23] – and mucins cover and protect the sinus and mouth epithelia[24] – and is also able to invade epithelial cells[25]. The presence of *H. pylori* in the sinuses could then be regarded as a biomarker of the extent of LPR in the upper

airway tract. However, at this time, we have no definitive proof of this, and we simply cannot rule out the possibility that the bacterium may use, in alternative or in conjunction, other routes to have its DNA reach the sinuses, for instance, via lymphatic or vascular transmission, from either the stomach or any other extra-digestive site.

Also, the presence of *H. pylori* in the sinuses apparently does not support a local pathogenic role does not entirely rule out the possibility that the bacterium may influence the course of an inflammatory disease of the sinuses. *H. pylori* gastric infection is known to cause a vast array of systemic effects, including a strong immunologic response and gastrin and cytokine release from the stomach mucosa, all of which may indirectly affect chronic inflammation in any part of the respiratory system[2]. Definitive proof of the clinical relevance of these *H. pylori*–induced systemic effects is, however, still lacking.

Conclusion

Our results suggest that, regardless of how *H. pylori* reaches the sinuses, its presence there does not seem to contribute to the local inflammatory status of the respiratory mucosa. The fact that *H. pylori* could not be cultured from nose samples and is only present in its DNA form, suggests either a transient presence of parts of its genome in the sinuses, or, instead, what could be seen as a defensive adaptive reaction in preparation for a more or less lengthy stay at an inhospitable location, a change to a viable non-culturable form, from which *H. pylori* could hypothetically regain activity, to either play a role in the oral-oral route of transmission or in an eventual gastric re-infection.

Acknowledgements

We thank Dr. Sérgio Dinis for assisting with the statistical analysis, Dr[a]. Mónica Oleastro for confirming the PCR results, and Dr[a]. Patrícia Fonseca for reviewing the manuscript.

Authorship contribution

PBD: Concept and design, data acquisition and analysis, drafting and final approval of the manuscript, accountability for all aspects of the work. TM: Data acquisition and analysis, final approval of the manuscript. MS: Data acquisition and analysis, draft revision, final approval of the manuscript. PLA: Data acquisition and analysis, draft revision, final approval of the manuscript. JV: Data acquisition and analysis, final approval of the manuscript. AMC: Data acquisition and analysis, draft revision, final approval of the manuscript. JV: Concept and design, data acquisition and analysis, drafting, final approval of the manuscript, accountability for all aspects of the work.

Conflict of interest

The Jorge Vitor's lab has received funding from New England Biolabs Inc., USA, since 1995. All the other authors have no conflicts of interest to disclose.

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